ÄKTAcrossflow™

User manual

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Table of Contents

1 Introduction

About this manual

The ÄKTAcrossflow™ *User Manual* provides instructions for performing crossflow filtration, also called tangential flow filtration, using the automatic ÄKTAcrossflow instrument and UNICORN™ software.

In addition to this manual, all users must also read the entire contents of the *ÄKTAcrossflow Operating Instructions* before installing, operating or maintaining the ÄKTAcrossflow system.

In this chapter

1.1 Abbreviations and terminology

This section explains the abbreviations that appear in the user documentation for ÄKTAcrossflow.

Term	Explanation
Cartridge	Also referred to as “Module” or “Cassette”; the unit encapsulating the membrane. The membrane can have different formats, such as flat-sheet cassette or hollow fiber cartridge.
Flat sheet cassette	Example of a unit encapsulating the membrane. A cassette has at least one inlet (feed) and two outlets (retentate and permeate).
Hollow fiber	Example of a unit encapsulating the membrane, with a tube-like structure made from a membrane and sealed inside a crossflow cartridge, with one inlet (feed) and two outlets (retentate and permeate).
Crossflow	Also called tangential flow filtration. In crossflow filtration, the feed solution flows parallel to the surface of the membrane. Driven by pressure, some of the feed solution passes through the membrane filter. Most of the solution is circulated back to the feed tank or reservoir. The movement of the feed solution parallel to the membrane surface helps to remove the buildup of foulants on the surface.
Cut-off	The effective pore size of the membrane. This is given in MWCO (molecular weight cutoff) for ultrafilters and µm for microfilters. The MWCO size designation for ultrafilters is given in Daltons (D or Da) or kiloDaltons (kD or kDa), referring to the molecular weight of an ideal globular protein that is 90% retained by the membrane. No industry standard exists; hence the MWCO ratings of different manufacturers are not always comparable.
Retentate	The portion of the feed solution that does not pass through a crossflow membrane filter and is returned to the feed tank or reservoir.
Permeate	Also called the filtrate. The portion of a process fluid that passes through a membrane.

1.2 Important user information

Read this before operating the product

All users must read the entire *Operating Instructions* before installing, operating or maintaining the product.

Always keep the *Operating Instructions* at hand when operating the product.

Do not operate the product in any other way than described in the user documentation. If you do, you may be exposed to hazards that can lead to personal injury and you may cause damage to the equipment.

Intended use

ÄKTAcrossflow is intended for research use only, and shall not be used in any clinical procedures, or for diagnostic purposes.

Safety notices

This user documentation contains WARNINGS, CAUTIONS and NOTICES concerning the safe use of the product. See definitions below.

Warnings

WARNING

WARNING indicates a hazardous situation which, if not avoided, could result in death or serious injury. It is important not to proceed until all stated conditions are met and clearly understood.

Cautions

CAUTION

CAUTION indicates a hazardous situation which, if not avoided, could result in minor or moderate injury. It is important not to proceed until all stated conditions are met and clearly understood.

Notices

NOTICE

NOTICE indicates instructions that must be followed to avoid damage to the product or other equipment.

Notes and tips

Note: *A note is used to indicate information that is important for trouble-free and optimal use of the product.*

Tip: *A tip contains useful information that can improve or optimize your procedures.*

Typographical conventions

Software items are identified in the text by ***bold italic*** text. A colon separates menu levels, thus ***File*** →***Open*** refers to the ***Open*** command in the ***File*** menu.

Hardware items are identified in the text by **bold** text (e.g., **Power** switch).

2 ÄKTAcrossflow applications and crossflow processes

About this chapter

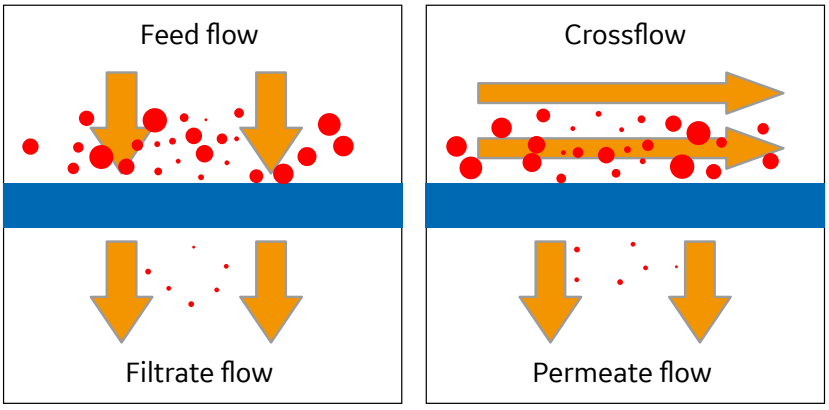
This chapter describes ÄKTAcrossflow applications and the principles of crossflow filtration.

In this chapter

2.1 Principles of crossflow filtration

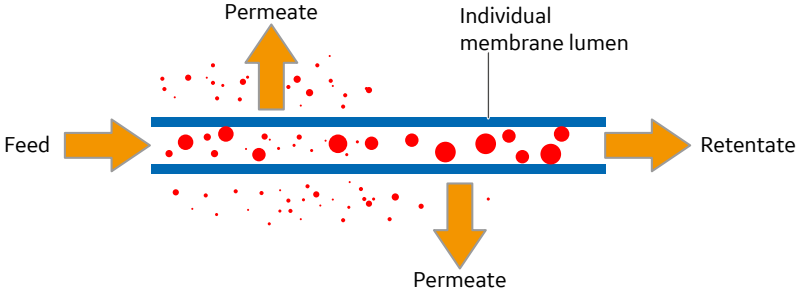
Normal flow filtration versus crossflow filtration

The ÄKTAcrossflow system is designed for crossflow (also known as tangential flow or "TFF") operation. Unlike normal flow filtration, or dead-ended filtration, crossflow methodology continuously sweeps the membrane surface by recirculating the feed flow parallel to the surface. This sweeping action minimizes blinding of the membrane and promotes consistent, long-term productivity. It also allows units to be cleaned, stored, and re-used as needed. The illustration below shows the difference between normal flow filtration and crossflow filtration.



System flows

As the feed is pumped through the cartridge, the retentate (the material excluded by the membrane pores) continues through the recirculation loop. The permeate, including solvent and solutes, is transported through the membrane pores and is collected separately. The illustration below shows the principle of crossflow filtration.



Filtration effects

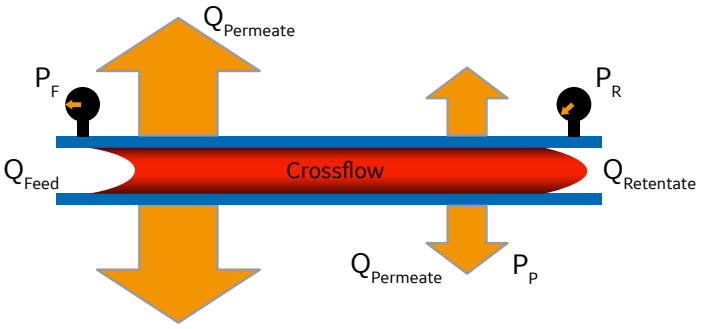
Term	Definition
Q_F, Q_R, Q_P	Feed, retentate, and permeate flow, respectively (mL/min)
P_F, P_R, P_P	Feed, retentate, and permeate pressure, respectively (bar or psi)

The filtration effect in crossflow filtration is a result of the applied "transmembrane pressure" (TMP).

$$TMP = \frac{P_F + P_R}{2} - P_P$$

The deltaP (ΔP) is the pressure drop between the feed (inlet) and the retentate (outlet) of the cartridge:$\Delta P = P_F - P_R$

The crossflow is proportional to the ΔP, if the permeate flow is zero.



2.2 ÄKTAcrossflow applications

About this section

This section describes the processes of Ultrafiltration and Microfiltration.

In this section

2.2.1 Ultrafiltration

Ultrafiltration (UF) is a pressure-driven, convective process that uses semipermeable membranes to separate species by molecular size or shape. Removing solvent from the solution results in solute concentration or enrichment. In ultrafiltration, species that are smaller than the membrane pores pass through the membrane while species that are larger are retained. UF membranes may also be used for diafiltration (buffer exchange) to remove salts or other microspecies from a solution via continuous dilution and re-concentration. The typical membrane cutoffs used in UF are 1 to 1000 to kD.

Concentration

A concentration step uses ultrafiltration membranes to reduce the volume of sample in the reservoir. The target product, such as protein, is retained at the retentate side of the membrane.

If the sample volume is larger than the reservoir volume, the reservoir can be continuously fed with sample solution (“fed batch” concentration).

	Ultrafiltration
Retentate	Proteins
Permeate	Small peptides and salts
Cutoff	1 to 1000 to kD

Diafiltration

A diafiltration step uses ultrafiltration membranes to remove salts or other microsolutes from a solution. Small molecules are separated from a solution while larger molecules are retained in the retentate. Diafiltration is often also called buffer exchange, whereby the feed solution is continuously, or repeatedly, filled up with a buffer. One buffer is removed and replaced with an alternative buffer.

A diafiltration or buffer exchange is typically run after a UF concentrating step using the same filter as for the concentration step. The product is retained at the retentate side, in the reservoir. Typical membrane cutoffs used for a diafiltration are 1 to 1000 to kD.

	Ultrafiltration
Retentate	Proteins (in new buffer)
Permeate	Small peptides and salts (old buffer)
Cut-off	1 to 1000 to kD

2.2.2 Microfiltration

Microfiltration is a pressure driven convective process, intended to separate larger insoluble particles (submicron size species) resulting in solution concentration or clarification.

Microfiltration is usually an upstream recovery process where cells and cell debris are separated from the other components in the solution, such as recombinant proteins. The product can be the cells in the retentate or the clarified protein solution in the permeate.

Typical cut-offs are 0.1 µm to 10 µm.

	Microfiltration
Retentate	Intact cells, cell debris
Permeate	Colloidal material, viruses, proteins, and salts
Cut-off	0.1 µm to 10 µm

Cell harvesting

Cell harvesting is the process of concentrating or dewatering the cell mass after fermentation. With cell harvesting, the cells are the target material and are recovered as product in the retentate.

The concentration factor that can be achieved is based on the starting concentration which can, in the case of yeast cells, be as high as 70% to 80% by cell weight. Typical concetration factors are as follows:

- *E. coli* cells: 5× concentration
- Yeast cells: 2× concentration
- Mammalian cells: 10× to 20× concentration

Cell washing

The cells can also be prepared by diafiltration or washing, for example, for transfer into a specific buffer for further processing, such as freezing or lysing. A cell washing step can be performed together with cell harvesting.

The washing process is commonly a constant volume diafiltration process, in which buffer is added to the cell suspension at the same rate as the permeate flow. After washing, the ideal end product would consist of the concentrated cells suspended in the buffer used to wash the cells. However, in practice the harvested cells and buffer can contain varying levels of unwanted elements such as precipitated proteins, enzymes, and cell debris.

Lysate clarification

In a clarification step, cells, cell debris, or other insoluble matter are retained by the membrane and the target product passes through the membrane into the permeate. Clarification is often performed on lysed cells (lysate) to separate the cell debris from the target product (usually recombinant proteins). Together with lysate clarification, a diafiltration step can be performed to maximize target product recovery from the lysate.

Membrane and cartridge selection

In cell harvesting, microfiltration membranes will easily retain all cells. The key to membrane selection is not based on retention, but on process optimization. For example, smaller pore size membranes often provide the highest permeate flux once the system is in a steady state. For more information on cartridge and tubing specifications, see *Section B Tubing specifications, on page 334*.

2.3 The ÄKTAcrossflow system

About this section

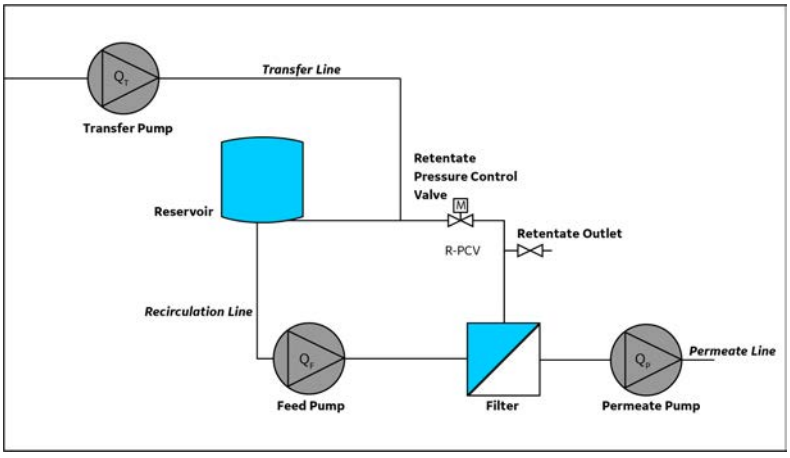
This section describes the ÄKTAcrossflow system including the flow path, the sensors and valves along the flow path, and the different control modes.

In this section

2.3.1 Description of system operation

In the ÄKTAcrossflow system, the sample solution is loaded into the reservoir with the transfer pump. The feed pump transports the solution to the filter unit. The flow through the membrane is regulated by adjusting the transmembrane pressure via the retentate pressure control valve (**R-PCV**). The remaining retentate flow, flowing parallel to the membrane surface, is recirculated back to the reservoir. During a concentration step, the volume in the reservoir is allowed to decrease. If the entire feed volume does not fit into the reservoir at the start of the concentration step, the transfer pump continually transports extra feed material to the reservoir. This maintains a constant retentate volume. During a diafiltration step, the transfer pump transports the new buffer to the reservoir continually to maintain a constant retentate volume.

Flow path overview



2.3.2 Control modes

Terminology

Term	Definition
Q_F, Q_R, Q_P	Feed, retentate, and permeate **flow**, respectively (mL/min)
P_F, P_R, P_P	Feed, retentate, and permeate **pressure**, respectively (bar)
TMP	Transmembrane pressure (bar or psi) $TMP = \frac{P_F + P_R}{2} - P_P$
DeltaP	Pressure drop (bar):$\Delta P = P_F - P_R$
Flux (LMH)	Permeate flow per membrane area:[l/h*m^2 or LMH]Q_P = Permeate flow (l/h)A = Membrane area (m^2)Flux =
Shear rate (sec^{-1})	Q_F = Feed flow (mL/min)n = Number of fibersr = Fiber radius (mm)Shear = $169.76527 \times Q_F/n \times r^3$

There are four filtration control modes on the ÄKTAcrossflow system:

- TMP control mode
- Flux control mode
- Permeate unrestricted flow
- Normal flow filtration

TMP Control mode

Control Mode:TMP Control	Control Element:		
	Feed Pump	Permeate Pump	R-PCV
TMP control with constant feed flowrate	$Q_F > 0$	Offset	TMP
TMP control with constant retentate flowrate	$Q_R > 0$	Offset	TMP
TMP control with constant DeltaP	$P_F - P_R > 0$	Offset	TMP

TMP control is usually used in ultrafiltration where the system must force the small components in the feed through the relatively small pores of the membrane. The control mode keeps one of the following options constant:

- constant feed flow
- constant retentate flow
- constant shear rate (hollow fibers only)
- constant deltaP

The TMP is mainly controlled by the retentate pressure control valve (**R-PCV**). The TMP control mode adjusts the retentate control valve and permeate pump to maintain the chosen TMP. Before the activation of TMP control, deltaP must be stable.

During TMP control, the permeate pump acts as a flow meter, with a pressure offset of 0.2 bar created by the permeate pressure control valve (**P-PCV**). This offset pressure is used to avoid low or negative pressure on the permeate side, which may affect the permeate pump's function as a flow meter.

If TMP < ΔP/2, the permeate pump offset will automatically be changed. The TMP control mode resets the other filtration control modes.

Note: *The TMP must be optimized for different applications (filter and sample).*

Flux control mode

Control Mode:flux Control	Control Element:		
	Feed Pump	Permeate Pump	R-PCV
Flux control with constant feed flowrate	$Q_F > 0$	Flux > 0	Offset, unrestricted for P_P > offset
Flux control with constant retentate flowrate	$Q_R > 0$	Flux > 0	Offset, unrestricted for P_P > offset
Flux control with constant shear rate (hollow fibers only)	shear rate > 0	Flux > 0	Offset, unrestricted for P_P > offset
Flux control with constant deltaP	$P_F - P_R > 0$	Flux > 0	Offset, unrestricted for P_P > offset

Flux control is usually used in microfiltration processes where the system restricts the permeate flow through the relatively large pores of the membrane to prevent rapid blinding of the membrane. The control mode keeps one of the following options constant:

- constant feed flow,
- constant retentate flow,
- constant shear rate (hollow fibers only),
- constant deltaP

In this mode, the TMP value is a function of the permeate flux.

If the permeate pressure is < 0.2 bar, the **R-PCV** closes to increase the retentate pressure. When the permeate pressure is above 0.2 bar, the permeate pump can start. A constant ramping during 60 sec. from flux 0 to the flux set point is performed. During the ramping, the function for the **R-PCV** is to maintain the permeate pressure above 0.2 bar.

Note: *If the permeate pressure drops below 0.2 bar during the process, the* **R-PCV** *valve will close to increase the permeate flow rate. A TMP limit alarm can be set. If the system reaches the TMP limit when active, the system will pause.*

Flux control resets the other filtration control modes.

Note: *The flux control rate must be optimized for difference applications (filter and sample).*

Permeate unrestricted flow

Control Mode:permeate unrestricted flow	Control Element:		
	Feed Pump	Permeate Pump	R-PCV
With constant feed flowrate	$Q_F > 0$	$P_R = P_P$ (≥ = offset)	P_R
With constant retentate flowrate	$Q_R > 0$	$P_R = P_P$ (≥ = offset)	P_R
With constant shear rate (hollow fibers only)	shear rate > 0	$P_R = P_P$ (≥ = offset)	P_R
With constant deltaP	$P_F - P_R > 0$	$P_R = P_P$ (≥ = offset)	P_R

Permeate Unrestricted Flow (PUF) is a filtration control mode designed to mimic a manual system, in which the P_R and $P_P = 0$. PUF starts the flow on the permeate pump at the offset permeate pressure (0.2 bar default). If the retentate pressure > offset, the retentate pressure value is used as a new offset. If retentate pressures < offset, the retentate control valve lifts the retentate pressure to the offset value.

PUF is typically used only for nonproduct steps, for example, in the Method Wizard created preproduct method (see *Chapter 6 Create preproduct steps using the Method Wizard, on page 92*)

Permeate unrestricted flow resets the other filtration control modes.

Normal flow filtration

In normal flow filtration mode, the flow and pressure over the filter are controlled by the feed pump only. In the permeate line, the permeate pressure control valve (**P-PCV**) is fully opened. Because of this, liquid can move through the permeate pump even though the check valves of the permeate pump are not active. To allow for a homogeneous flushing of the permeate pump heads during normal flow filtration, the permeate pump is running idle at a low flow rate (20% of the feed flow rate) while liquid is transferred by the feed pump.

The pressure over the normal flow filter (pNFF) is the difference between the feed pressure and the permeate pressure.

pNFF = feed pressure - permeate pressure

Control Mode:normal flow filtration	**Control Element:**		
	Feed Pump	**Permeate Pump**	**R-PCV**
NFF control with constant feed flow	100%	20% of feed flow	R-PCV: closed P-PCV: open
NFF control with constant feed pressure	Pump ramps up to desired feed pressure set point (pNFF)	20% of feed flow	R-PCV: closed P-PCV: open

The normal flow filtration control mode is used to test the clean water flux or normalized water filtration (NWF) for hollow fibers with a pore size cut off of 0.1 µm and larger.

2.3.3 Sensors and valves

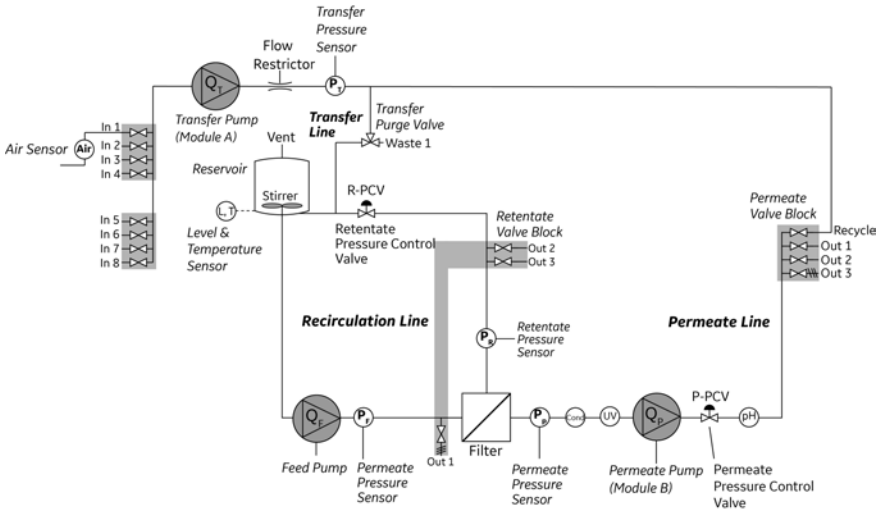
The system contains several valves and sensors. There are sensors for:

- pressure
- conductivity
- UV absorbance
- pH
- temperature
- reservoir level
- air

The output from the sensors are monitored by the UNICORN control software during the process run.

The sensors and valves are described in *Chapter 15 System components, on page 317*

Detailed illustration of the system flow path



2.4 Filters

The ÄKTAcrossflow system supports the two most common crossflow filter designs, hollow fibers and flat sheet cassettes, with surface areas suitable for flow rates up to 600 mL/min.

Hollow fibre filters

GE manufactures a complete selection of crossflow ultrafiltration and microfiltration hollow fiber membranes. In addition, the Start AXM and AXH hollow fiber cartridges, with a surface area of 40 or 50 cm^2, are especially designed for the ÄKTAcrossflow instrument. These cartridges are configured for convenient linear scaling and to optimize any candidate application around reproducible and predictable fluid mechanics.

In the hollow fiber cartridge, the tube-like structure is made from a membrane and sealed inside a crossflow cartridge. When in use, the feed stream flows into the inner diameter of one end of the hollow fiber and the retentate flows out the other end. The permeate passes through the membrane (the walls of the hollow fiber) and is routed out of ports at the side of the cartridge.

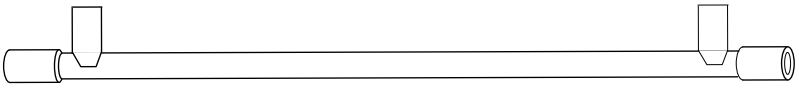


Figure 2.1: Schematic of a hollow fiber filter

Hollow fibre filters with UNF fittings recommended for use with ÄKTAcrossflow are shown in the following table.

For assembling instructions and ordering information, see the *ÄKTAcrossflow Operating Instructions*.

Flat sheet cassettes

In addition to hollow fiber cartridges, the ÄKTAcrossflow system has been designed to work with flat sheet crossflow filter cassettes from a variety of manufacturers, with surface areas up to approximately 1000 cm^2.

2.5 Associated documentation

For a list of all associated system-specific and software documentation, refer to the *ÄKTAcrossflow Operating Instructions*.

3 System Preparation

About this chapter

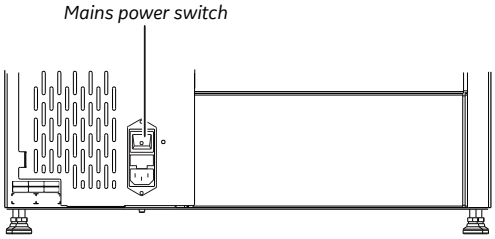
This chapter contains information for preparing the ÄKTAcrossflow system and connecting it to UNICORN, as well as how to calibrate component parts.

In this chapter

3.1 Start the instrument

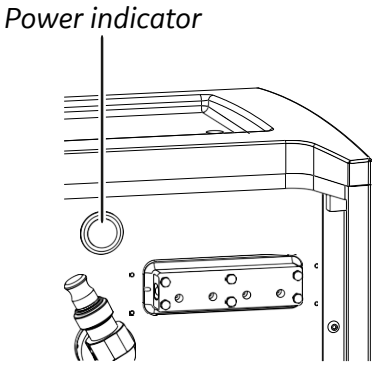
To start the ÄKTAcrossflow system:

Step	Action
1	Switch on the instrument at the mains power switch located on the rear panel.



Note:

The **Power indicator** *on the front panel flashes slowly until the internal communication with the CU (Control Unit) is established.*



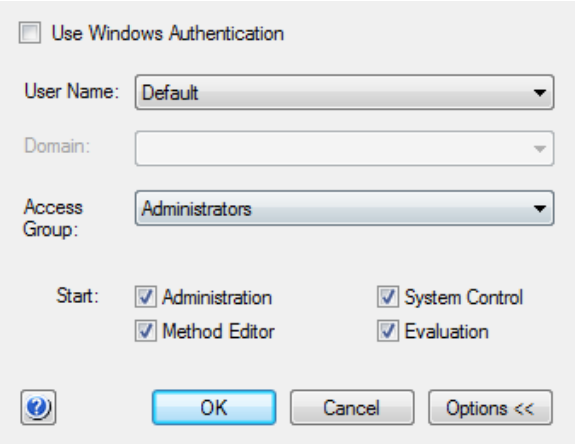
2	Switch on the power to the PC and the monitor.

3.2 Start UNICORN and connect to the instrument

Follow the instructions to start UNICORN and log on to the program. A valid e-license must be available for the workstation. See *UNICORN Administration and Technical Manual* for more information about e-licenses.

Step	Action
1	Double-click the UNICORN icon on the desktop.  *Result:* The ***Log On*** dialog box opens. ***Note:*** *If there is no connection to the database, it is still possible to log on to UNICORN and control a running system. The* ***Log On*** *dialog box will give the option to start* ***System Control*** *without a database. Click* ***Start System Control*** *to proceed to the next* ***Log On*** *dialog box.*

Step	Action
2	In the ***Log On*** dialog box: **a.** Login as default user with password ***default***.



Either

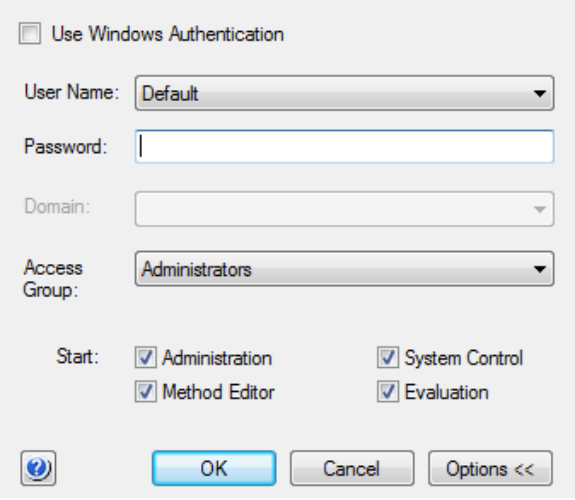
- Click a user name in the ***User Name*** list and enter the password in the ***Password*** field.

  Note:

  It is also possible to select the ***Use Windows Authentication*** *check box and enter a network ID in the* ***User Name*** *box.*

or

Step	Action



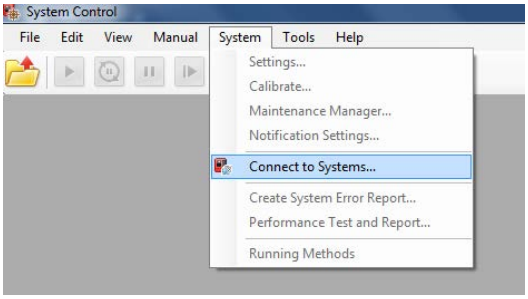
3 Select which UNICORN modules to start.

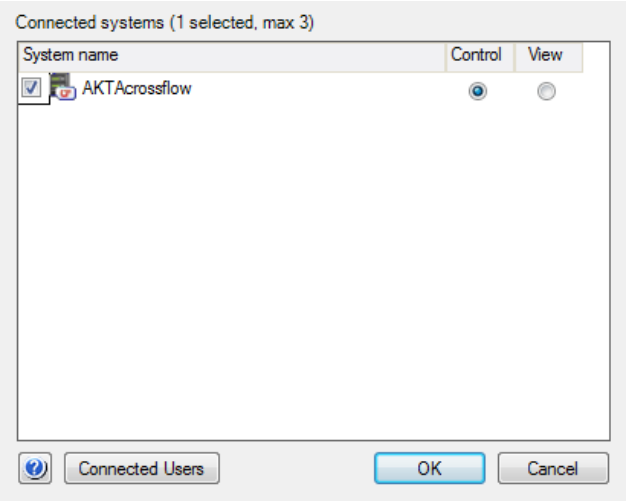
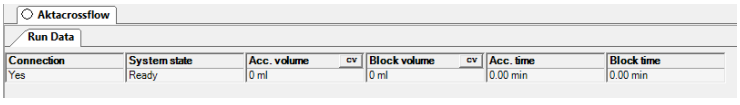
4 Click ***OK***.

Result:

The UNICORN modules open.

Follow the instructions to connect the instrument to UNICORN.

Step	Action
1	In the ***System Control*** module either: • Click on the ***Connect to Systems*** button,  *or* • click ***Connect to Systems*** on the ***System*** menu.  *Result:*The ***Connect to Systems*** dialog box opens.

Step	Action
2	In the ***Connect to systems*** dialog box: • Select the checkbox in front of the system name. • To control the selected system, click ***Control***. ***Note:*** *Instruments that are turned off or disconnected from the network appear dimmed and cannot be connected.*  ***Tip:*** *To view the users currently connected to systems, either in control or view* *mode, click the* ***Connected Users*** *button.*
3	When the communication between UNICORN and the instrument unit is established: • There is a constant light on the **Power** indicator on the instrument unit. • The ***Connection*** box shows ***Yes***. • The ***System state*** box shows ***Ready***. 

3.3 Assembling filters

Prepare the filter for use

- UltrafiltrationUF filter units are shipped with an appropriate storage solution within the pore structure to prevent drying of the membrane (see filter manufacturer for details). GE hollow fiber ultrafilter cartridges are shipped in an isopropanol/glycerol solution. This mixture enhances wetting but may cause the fibers to appear wavy. Trace amounts of isopropanol may remain when the cartridges are shipped.

  The glycerol must be thoroughly rinsed from the cartridge prior to use. In addition to preventing drying, the glycerol minimizes entrained air within the pore structure of the membrane wall which may become “locked-in”, reducing permeability until the air has been displaced by liquid.

- MicrofiltrationAlthough MF membrane cartridges are shipped dry, without preservative solutions (such as GE hollow fiber microfilter cartridges), it is recommended to rinse cartridges before first process exposure or heat sterilization.

For instructions on cleaning a hollow fiber filter cartridge and system prior to a filtration run, refer to *Chapter 6 Create preproduct steps using the Method Wizard, on page 92*.

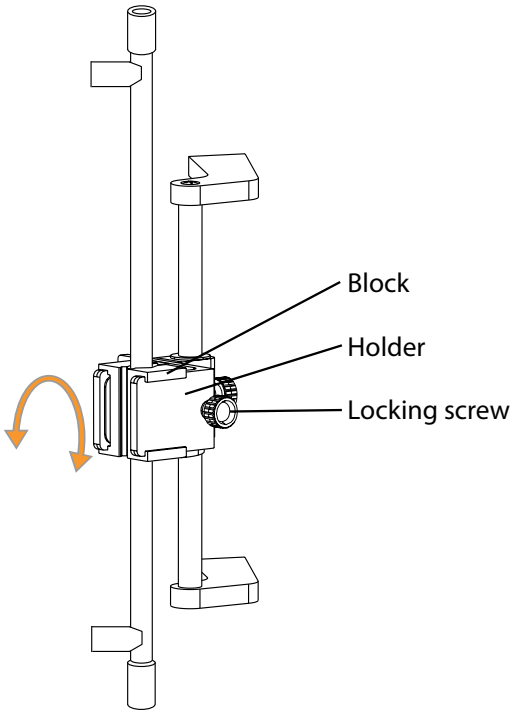
Connect the Start AXM hollow fibre cartridge

The Start AXM hollow fiber cartridge can be installed by connecting it to the hollow fiber cartridge holder.

The holder is spring-loaded, and can be rotated 360° without loosening any screws. It has fixed positions separated by 90°, (i.e., vertical or horizontal position). The holder allows cartridge diameters from 3 to 23 mm.

Install the filter on the instrument unit as follows:

Step	Action
1	Attach the block to the holder.

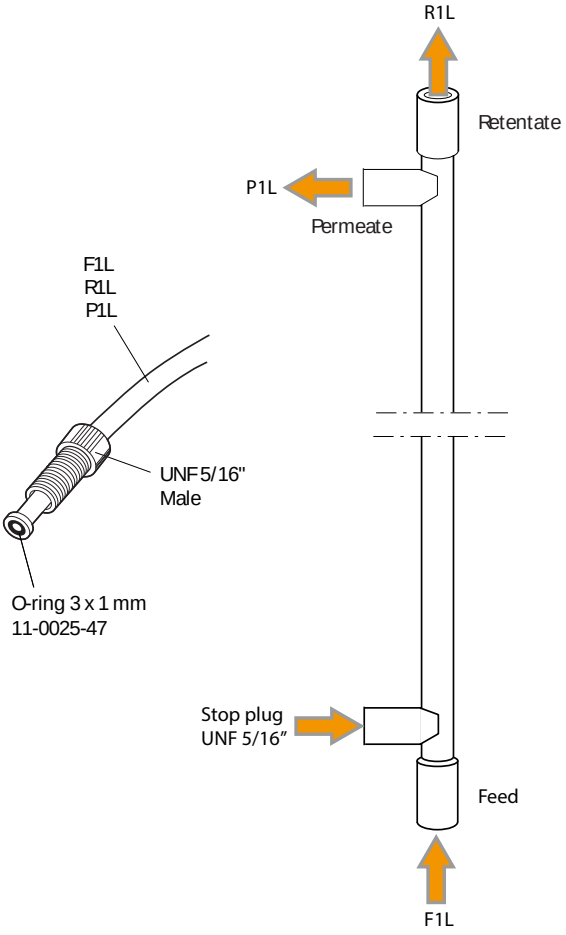


2	Carefully attach the cartridge in the holder by tightening the locking screw.

Connect tubing to the hollow fibre cartridge

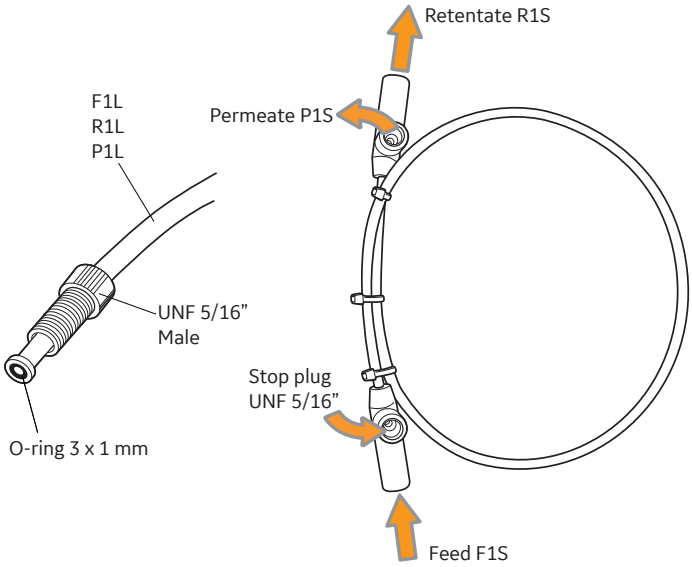
Connect the cartridge to the retentate, permeate and feed lines.

Note: *To minimize holdup and working volume, the recommended tubing length and diameter for use with the Start AXM hollow fiber cartridge, refer to Appendix A.*


R1L
Retentate
P1L
Permeate
F1L
R1L
P1L
UNF 5/16"
Male
O-ring 3 x 1 mm
11-0025-47
Stop plug
UNF 5/16"
Feed
F1L

Connect the Start AXH cartridge

The Start AXH cartridge can be installed by connecting it to the retentate, permeate and feed lines, as shown in the illustration below. There is no need for a holder.



For details of recommended tubing, refer to *Appendix A*.

Connect a flat sheet cassette

For guidance on connecting a flat sheet cassette and holder, contact your local GE representative.

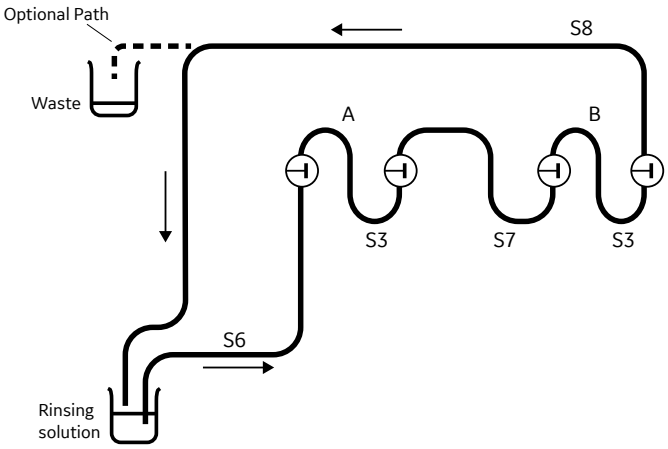
3.4 The pump rinsing system

Introduction

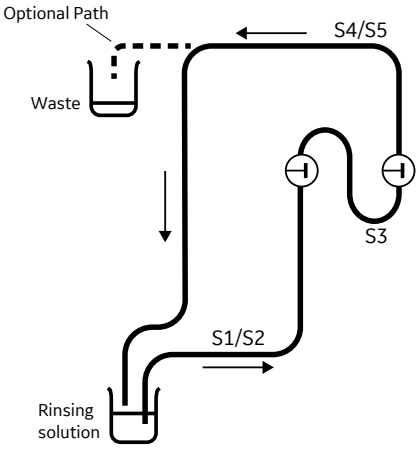
Leakage between the pump chamber and the pump drive mechanism is prevented by a seal that is continuously lubricated by the presence of eluent. To prevent any deposition of salts from aqueous buffers on the pump pistons and to prolong the life of the seals, the low pressure chamber behind the piston is flushed continuously with a low flow of 20% ethanol prepared in ultra pure water or equivalent.

The piston rinsing system tubing is connected to the rearmost ports on the pump heads. The following illustration shows the tubing configuration of the piston rinsing system.

Feed pump P-984



Transfer & Permeate pump P-982



Although the seal prevents leakage between the pump chamber and the drive mechanism, “active transport” or movement of a very small amount of liquid between the product to the rinse side is possible. To eliminate the risk of re-introducing proteins/cells into subsequent batch runs, replace the rinse solution with fresh solution either every day or between crossflow runs.When running the ***System Sanitization*** program, the rinsing solution should be exchanged with 1M NaOH, followed by careful rinsing with water and replacement of the solution with 20% ethanol.

The illustration below indicates the different components of the pump rinsing system.

Using the pump rinsing system

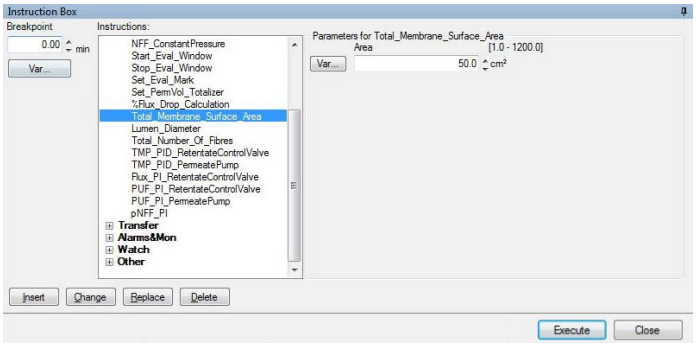
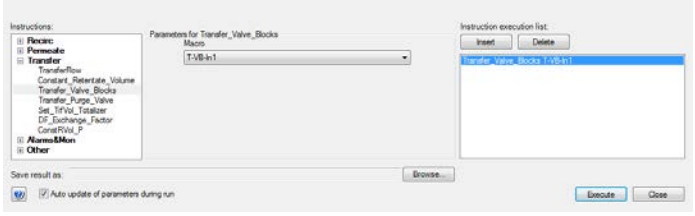
To use the piston rinsing system:

Step	Action
1	Fill the rinsing system bottles with 20% ethanol.
2	Insert the rinsing inlet and outlet tubing ends into the rinsing solution. ***Note:*** *To eliminate the risk of re-introducing bacteria or other contaminants, use the optional path with a separate waste bottle.*
3	Fill the tubing with solution using a syringe that is connected to the outlet tubing end.
4	Repeat steps 1 to 3 for all pumps.

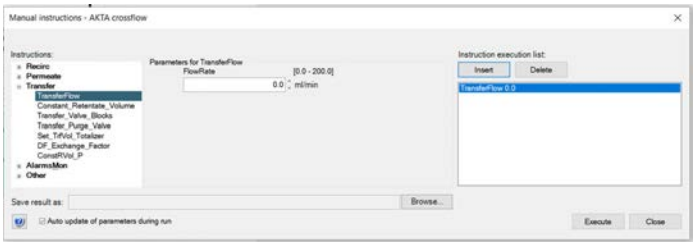
Cleaning the pump piston rinsing system

To clean the pump piston rinsing system use the following procedure

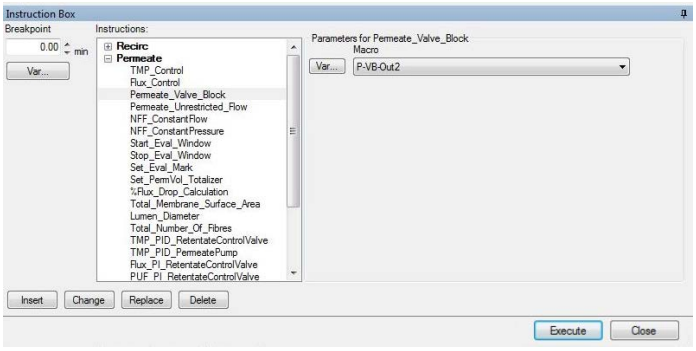
Step	Action
1	Remove the rinsing solution bottles and empty them. Place the tubing in waste containers.
2	Fill the bottles with freshly prepared rinsing solution (20% ethanol) and insert the rinsing system tubing.

Step	Action
3	Connect the **3-way connector** is connected to the **feed**, **retentate**, and **permeate** lines in place of a filter.
4	In ***System Control***, select ***Manual/Execute Manual Instructions***.
5	Under ***Permeate/Total_Membrane_Surface_Area***, set the membrane area to 50 cm^2 and click ***Execute***. 
6	Place **transfer inlet 1** tubing into a 500 mL bottle of distilled water. Under ***Manual***, select this port under ***Transfer/Transfer_Valve_Block***, and click ***Execute***. 
7	Start the **transfer pump** at a flow rate of 200 mL/min to bring distilled water into the reservoir. At the same time that the transfer flow starts, start a ***FeedFlow*** of 400 mL/min

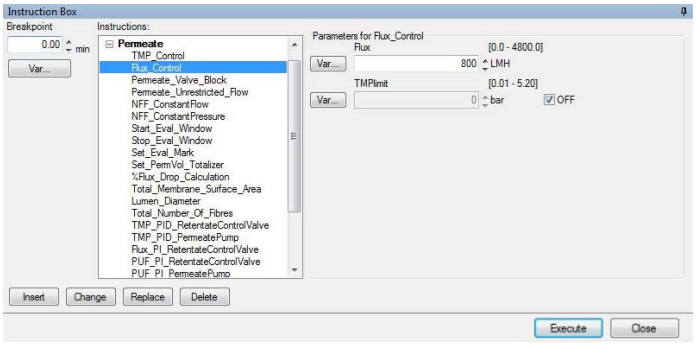
Step	Action
8	Stop the transfer pump after 1 minute when approximately 200 mL is in the reservoir.

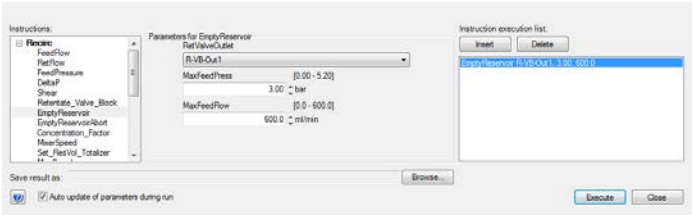


9	Open **permeate valve block out 2**.



10	Under ***Permeate/Flux_Control***, set a flux rate of 800 LMH and pump for two minutes.



Step	Action
11	Under ***Recirc/EmptyReservoir***, select **R-VB-Out1** as ***RetValveOutlet***, set ***MaxFeedPressure*** to 3.0 bar, and click ***Execute***.
	
12	The system will stop when the reservoir is empty.

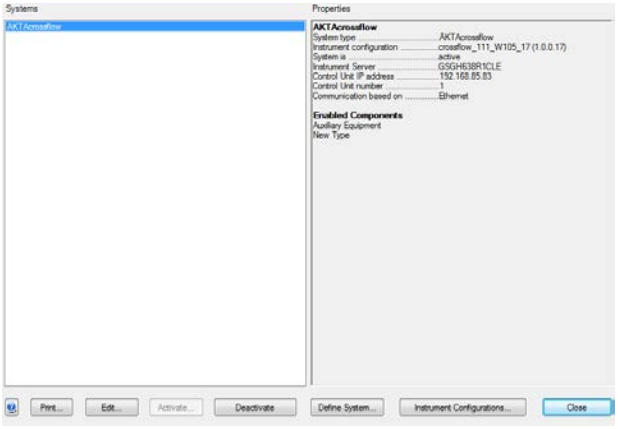
3.5 Selecting type of Retentate valve block

The **Retentate valve** block type, old type or new type, must be selected in UNICORN. If you are unsure which type of retentate valve block your system has, contact your local GE office for support.

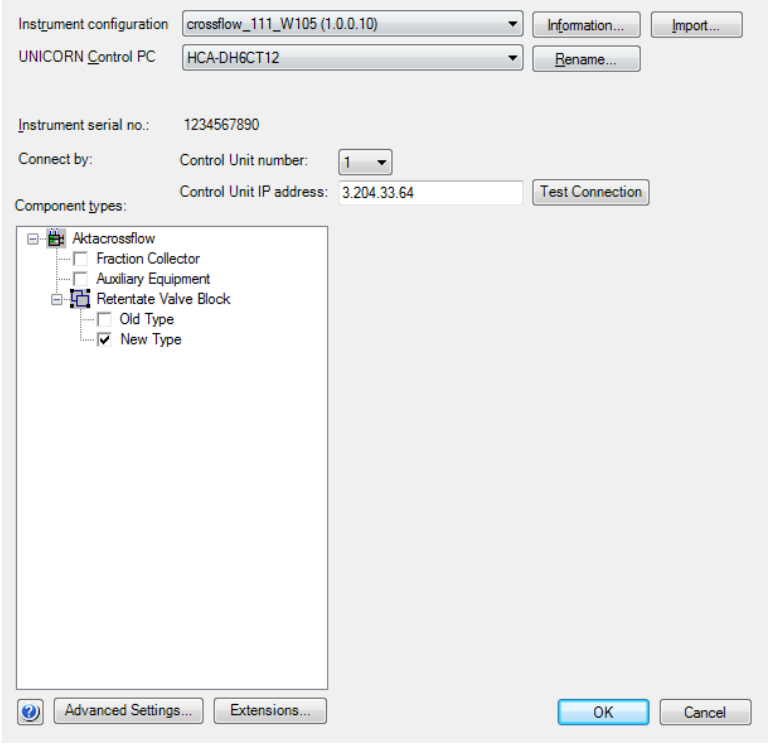
To select the **Retentate valve** block type, use the following procedure.

Step	Action
1	In ***UNICORN Administration***, select ***System Properties***.

Step	Action
2	Select a system and click ***Edit***.



3	Under ***Component*** types, select type of retentate valve block used from the following options. either ***old type*** or ***new type***.



Step	Action
4	Click ***OK*** to close the ***Edit System*** dialog.
5	Click ***Close*** to close the ***System Properties*** dialog.

3.6 Calibrate the level sensor

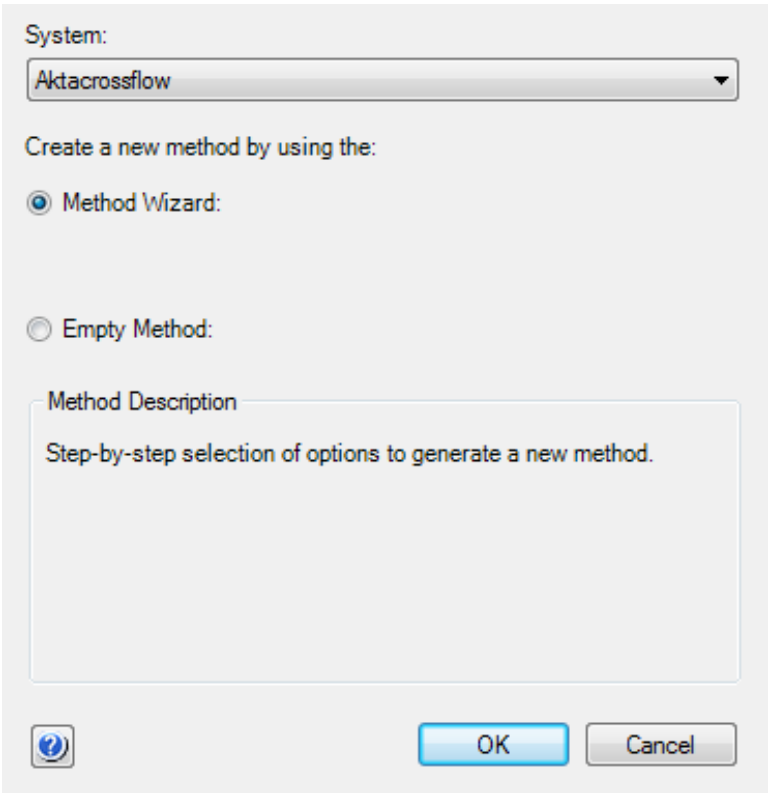
About this section

This section provides instructions on how to calibrate the level sensor using either the ***Method Wizard*** or by manually running the calibration program.

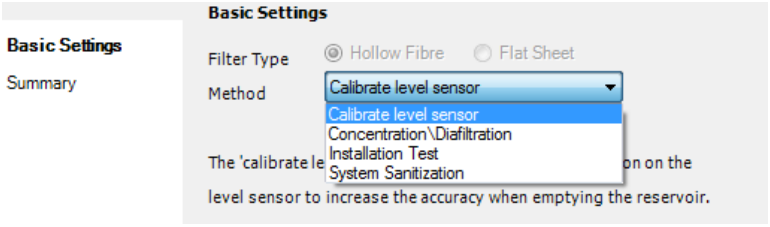
In this section

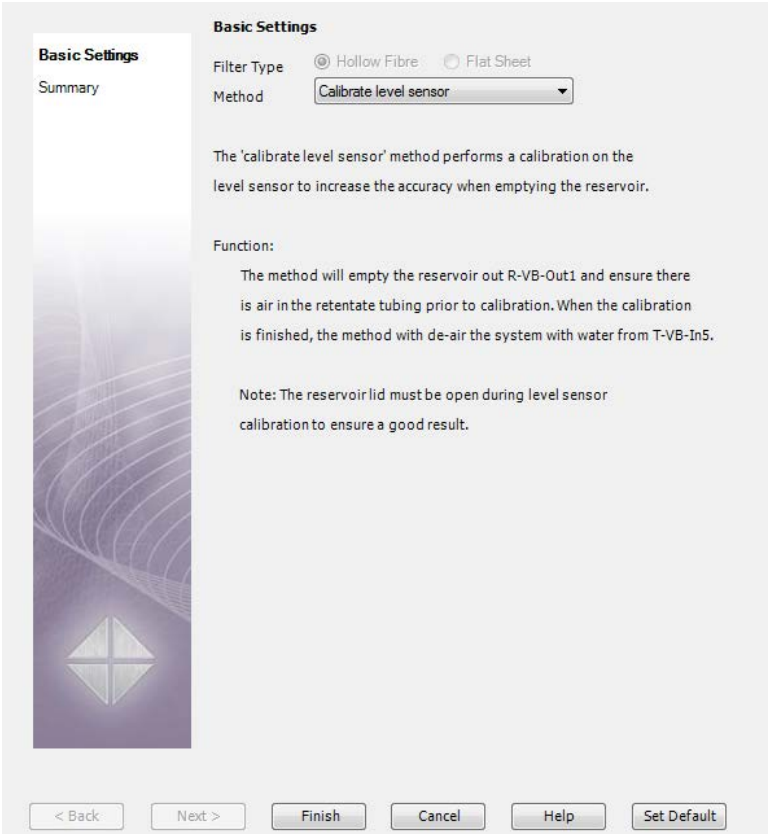
3.6.1 Calibrate the level sensor using the *Method Wizard*

The ***Method Wizard*** enables the creation of a method to automatically calibrate the level sensor, which sets the zero level on the reservoir. The calibration should be performed as needed, or when the level sensor is changed.

Step	Action
1	In the ***Method Editor*** click the ***Create New Method*** icon. 
2	In the ***System*** drop down menu, select ÄKTAcrossflow. 
3	Select ***Method Wizard***.
4	Click ***OK***.

Step	Action
5	In the ***Basic Settings*** dialog, select ***Calibrate level sensor*** under the ***Method*** drop down menu.





6	Click ***Finish***
7	To run the method, first save the method in ***Method Editor*** and then, in ***System Control***, run the method *Section 10.2 Start a run, on page 213*.

Note: *If the level sensor is out of calibration, this method may not properly calibrate the level sensor. At the end of the method, if the reservoir is not empty, it might be necessary to manually empty the reservoir and manually set the zero level on the sensor in* ***System Control****.*

3.6.2 Manual calibration of the level sensor

The reservoir level sensor can be calibrated using the Method Wizard or manually.

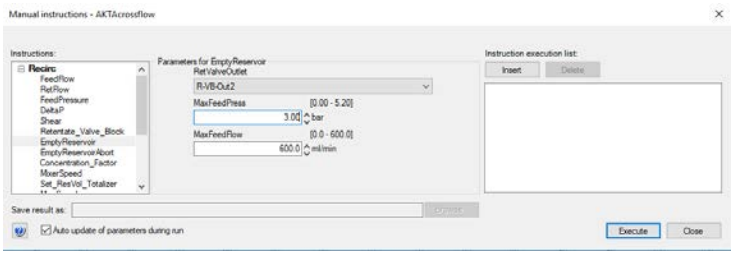
Using the Method Wizard to calibrate the level sensor is recommended because it reduces the risk of error and is more convenient. To calibrate the level sensor manually, use the following instructions:

Note: *When calibrating the level sensor, the liquid used must be at ambient temperature.*

Empty the reservoir

To manually calibrate the level sensor, the reservoir must first be emptied. To do this, use the following procedure.

Step	Action
1	Open the reservoir lid to allow air to flow freely into the reservoir.
2	In the ***System control*** dialog, select ***Manual*** →***Execute Manual Instructions***.
3	Under ***Recirc*** →***EmptyReservoir***, set ***RetVavleOutlet*** to ***R-VB-Out1*** and set ***MaxFeedPressure*** to 3.0 bar.



4	Click ***Execute***
5	Wait until the reservoir is emptied. ***Note:*** *The **EmptyReservoir** instruction empties the reservoir but not the small cavity in the bottom of the reservoir. This cavity must also be emptied before calibration.*
6	Under ***Recirc*** →***Retentate_Valve_Block***, select ***R-VB-Out1*** , then press ***Execute***.
7	Under ***Recirc*** →***FeedFlow***, enter 20 mL/min, then press ***Execute***.

Step	Action
8	Check that the cavity in the bottom of the reservoir is completely empty. Do not stop the flow.

Run level sensor calibration

To run the level sensor calibration, use the following procedure.

Step	Action
1	In the ***System Control*** module, select ***System*** →***Calibrate***. The ***Calibration*** dialog appears.
2	In the ***Monitor*** box, select ***ZeroLS***. Click the ***Start calibrate*** button to reset the level sensor reading to 0.
	CAUTION The reservoir level sensor is highly sensitive. Do not insert any objects into the cavity in the bottom end plate of the reservoir since this can damage the level sensor.
3	Under ***Manual*** →***Execute Manual Instructions***, select ***Recirc*** →***FeedFlow***.
4	Enter ***0 mL/min*** in the ***FeedFlow*** box to stop the feed flow, then click ***Execute***.
5	Under ***Recirc*** →***Retentate_Vavle_Block***, select ***R-VB-recycle*** and click ***Execute***.
6	Click ***Close***.
7	Manually fill the reservoir with 50 mL of distilled water.
8	Empty the reservoir by selecting ***Recirc*** →***EmptyReservoir*** →***R-VB-Out1***, and enter ***3*** in the ***MaxFeedPress*** box and ***600*** in the ***MaxFeedFlow*** box.
9	Click ***Execute***.
10	Click ***Close***.

3.7 Calibrate the transfer pressure sensor

In pressure sensor P_T, the zero pressure-reading can be calibrated. The amplification in sensors P_T, P_F, P_R, and P_P is already calibrated at the factory.

Calibration of the pressure sensor P_T

To calibrate the zero pressure-reading on the transfer pump, use the following instructions:

Step	Action
1	In the ***System Control*** module, under ***Manual*** →***Execute_Manual_Instructions***, select ***Transfer*** →***Transfer_Purge_Valve*** and set to ***Waste***. Click ***Execute***.
2	Under ***Transfer*** →***Transfer_Valve_Block 1***, select ***T-VB-In1***. Click ***Execute***.
3	Immerse the tubing from inlet ***T-VB-In1*** in distilled water.
4	In the ***System Control*** module, select ***System*** →***Calibrate***.*Result: the* ***Calibration*** dialog appears. *Result:* In the ***System Control*** module, select ***System*** →***Calibrate***. dialog appears.
5	In the ***Monitor*** box, select ***TrfPress***.
6	Click the ***Start calibrate*** button when the pressure is stable. The transfer pressure has now been set to 0.
7	Press ***Close*** to finish calibration.

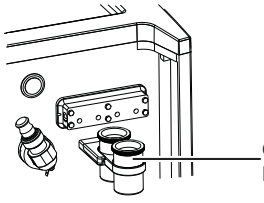
3.8 Calibrate the pH electrode

A good laboratory routine is to calibrate the pH electrode at least once a day, when the electrode is replaced, or if the ambient temperature is changed. The pH electrode is calibrated using standard buffer solutions in a two point calibration. The two buffer solutions may have any pH value as long as the difference between them is at least 1 pH unit, and the expected pH during the run is within this interval.

Note: *The pH-calibration kit can be found in the Accessory box delivered with the ÄKTAcrossflow.*

To calibrate the pH electrode, use the following procedure.

Step	Action
1	In the ***System Control*** module, select ***System*** →***Calibrate***.
2	Select ***pH*** from the ***Monitor*** dropdown menu in the ***Calibration*** window.
3	Prepare two reference buffer solutions, the first normally pH 7.0. The difference in pH value between them must be at least 1 pH unit. The expected pH value during the run should be within this interval.
4	Use the holder on the front panel for the reference buffer solution containers.



5	Remove the pH electrode from the cell holder and immerse the electrode in the first reference solution. ***Note:*** *To avoid leakage from the system after removing the pH electrode, replace it with the pH electrode dummy.*
6	Enter the known pH value of the solution in the ***Set the reference value*** field.

Step	Action
7	The pH reading is shown under ***Current value***. When the pH value has stabilized, click ***Read value 1***.
8	Rinse the electrode tip with distilled water and then immerse the electrode in the second reference solution (e.g., pH 4.0 or 9.0).
9	Enter the known pH value of the second reference solution in the second ***Set the reference value*** field.
10	When the pH value has stabilized, click ***Read value 2***. The calibration is finished.
11	After the calibration, values are automatically entered into the ***Calibrated electrode slope; %***and ***Asymmetry potential at pH7; mV*** fields.

A new electrode has a slope of typically 95% - 102% and an asymmetry potential within ±30 mV. As the electrode ages, the slope decreases and the asymmetry potential increases.

As a rule, when the ***Asymmetry potential at pH7; mV*** value is outside of ±60 mV and the ***Calibrated electrode slope; %*** value is lower than 80%, and no improvement can be achieved by cleaning, the electrode should be replaced.

An electrode is still usable at lower slopes and higher asymmetry potentials but the response will be slower and the accuracy diminished.

Before use, rinse the pH electrode using distilled water.

3.9 Calibrate the conductivity cell

Enter a new cell constant

After replacing the conductivity cell, the cell constant has to be set. The cell constant is shown on the packaging of the new cell.

Step	Action
1	In the ***System Control*** module, select ***System*** →***Calibrate*** and then ***Cond_Cell*** in the ***Monitor*** dropdown menu.
2	Enter the cell constant in the ***Set the reference value*** field.
3	Click ***Read value 1***. The new cell constant is updated. Click ***Close***.

Calibrate the temperature sensor

Calibration of the temperature sensor in the conductivity cell is only necessary when the cell is used in high accuracy measurements or if the cell is replaced.

Step	Action
1	Make sure that the conductivity cell together with a precision thermometer are not exposed to a draft. Leave them for 15 minutes to let the temperature stabilize.
2	In the ***System Control*** module, select ***System*** →***Calibrate*** and choose ***Cond_Temp*** in the ***Monitor*** dropdown menu.
3	Read the temperature on the thermometer.
4	Enter the temperature in the ***Set the reference value*** field.
5	Click the ***Read value 1*** button.

Set up conductivity temperature compensation

The conductivity of a buffer is temperature dependent. To relate conductivity to concentration and/or compare conductivity values, temperature compensation should be used. The compensation consists of a compensation factor together with a reference temperature. All conductivity values will then automatically be converted to the set reference temperature.

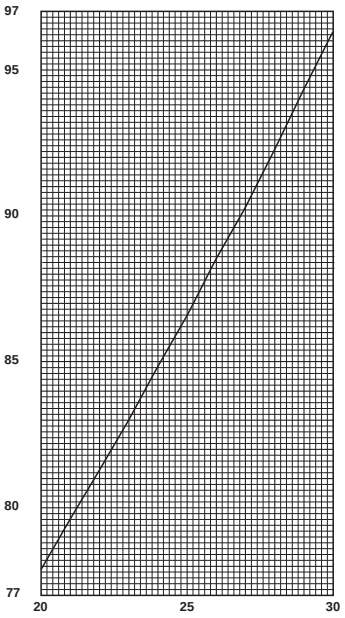
Step	Action
1	In the ***System Control*** module, select ***System*** →***Settings*** and click the ***Monitors*** button.
2	Choose the instruction ***CondTempComp***.
3	The factor is expressed in percentage increase of conductivity per °C increase in temperature. If the temperature compensation factor is unknown, a general approximate value of 2% can be set for many common salt buffers. If no temperature compensation is needed, enter the value 0% in the ***CompFactor*** field.
4	Choose the instruction ***CondRefTemp***.
5	Select the reference temperature to which the measured conductivity values will be converted (normally 20 or 25 °C).
6	Enter an appropriate temperature in the ***RefTemp*** field.
7	Click ***OK***.

Calibrate the cell constant

Normally it is not necessary to adjust the cell constant as the cell is precalibrated on delivery. Adjustment is only necessary when replacing the conductivity cell with a cell whose cell constant is unknown.

Note: *The conductivity temperature compensation must not be used when adjusting the cell constant. Set the compensation factor to 0 (see section Set up conductivity temperature compensation, on page 56). The temperature sensor must be calibrated before adjusting the cell constant (see section Calibrate the temperature sensor, on page 55).*

Step	Action
1	Prepare a calibration solution of 1.00 M NaCl, 58.44 g/L. Let the solution reach room temperature.

Step	Action
2	Fill the cell completely with the calibration solution by pumping at least 15 mL through the cell with a syringe.
3	When finished, wait for 15 minutes until the temperature is constant in the range of 20°C to 30 °C.
4	In the ***System Control*** module, select ***System/Calibrate***. Select ***Cond_Cal*** in the ***Monitor*** dropdown menu.
5	Read the conductivity value displayed under ***Current value*** and compare it with the theoretical value from the graph opposite at the temperature of the calibration solution. If the displayed value and the theoretical value correspond, no further action is required. If the values differ, proceed with steps 6, 7 and 8.
6	
7	Enter the theoretical conductivity value according to the graph in the ***Set the reference value*** field.
8	Click the ***Read value 1*** button. The new cell constant is automatically calculated and updated.

4 Handling methods in the ÄKTAcrossflow

About this chapter

This chapter describes how to create ÄKTAcrossflow methods in UNICORN.

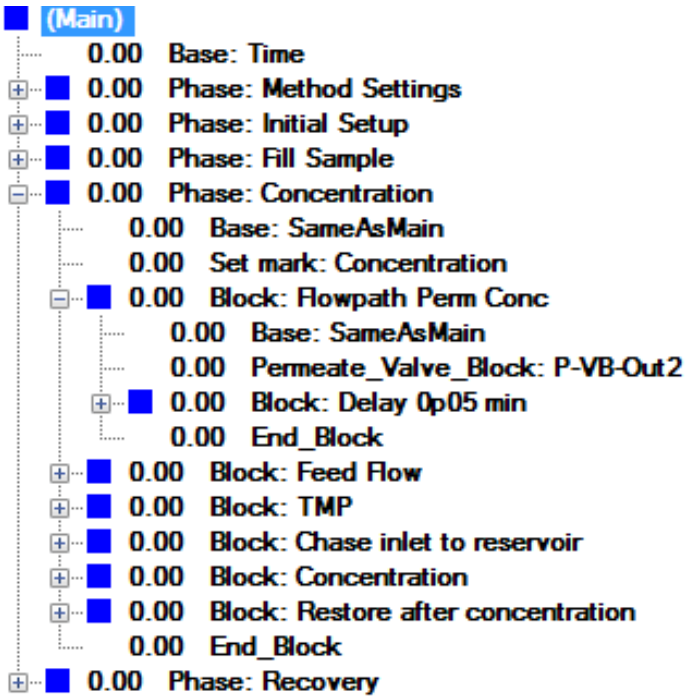
In this chapter

4.1 A UNICORN method

Phases and blocks

The ***Text pane*** in the ***Method Editor*** of UNICORN displays the method as a list of text instructions. The instructions are usually organized into phases, denoted by blue square symbols, for a specific functional use, for example to load a sample or to perform a concentration step. A phase can contain individual instructions or substructure in the form of blocks. The phases and blocks can be expanded to show the instructions within the block.

A method always starts with the ***Methods Settings*** phase.



To create a method, *either*

- use the ***Method Wizard creator***, see *Section 4.2.1 Create crossflow methods using the Method Wizard, on page 62* (recommended) *or*
- Use the ***User Defined*** phase in the ***Predefined Phase library*** to create new phases, see *Unicorn User Manual* for more information.

  Note: ***User Defined*** *is an empty phase designed for text editing methods. Such phases may be saved in the personal or global phase library for reuse in other methods.*

Base

Every phase and block must start with a ***Base*** instruction, which defines the parameter for calculating breakpoints. Different phases and blocks can use different bases. In the ÄKTAcrossflow, the base can be one of the following:

- ***Volume*** (mL)
- ***Time*** (minutes)
- ***SameAsMain*** (all blocks apart from the main block), which means that the block will use the base defined in the main block.

Note: *Do not use **Column Volume (CV)** as base in ÄKTAcrossflow methods as it is not relevant and will lead to incorrect methods.*

Calls

To execute the instructions contained within a phase or block in a method, the phase or block must be called by the program. When a phase or block is called, the instructions in the phase or block are executed in the order written until the phase or block ends, or the ***End_Block*** instruction is executed. There are two types of calls:

- Unconditional calls, which are made with a ***Phase*** or ***Block*** instruction.
- Conditional calls, which are made with a ***Watch*** instruction. ***Watch*** instructions allow a specified block or instruction to be called when a chosen monitor signal meets a given condition.

Watch and Hold_Until

The breakpoint when the ***Watch*** instruction is issued determines when the watch begins. A watch remains active until the condition is met or a new ***Watch*** instruction is issued for the same monitor. The watch is cancelled automatically when the condition is met. A watch can also be turned off with the ***Watch_off*** instruction.

The ***Hold_Until*** instruction is a special kind of ***Watch*** instruction. The method is put on hold until a specific condition is met (signal, test, or value), or the time-out is reached. Afterwards, the remaining instructions in the method are executed.

4.2 Creating a new method

About this section

This section describes how to create a new ÄKTAcrossflow method in UNICORN by using both the ***Method Wizard*** and the ***Text Instructions*** settings.

To create a new method, there are two alternatives:

- In the ***Method Wizard***, customized methods for most purposes are made by setting appropriate values for the method variables.
- In the ***Text Instructions*** editor in the ***Method Editor*** module, more advanced editing facilities are available.

In this section

4.2.1 Create crossflow methods using the Method Wizard

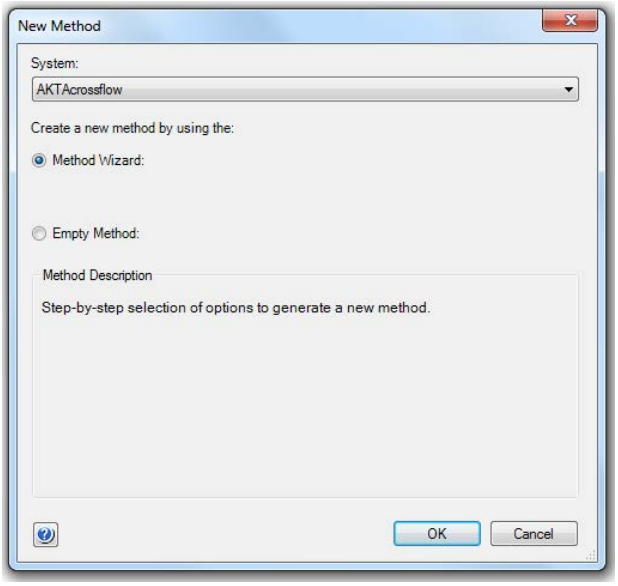
ÄKTAcrossflow methods are complex and include many steps which are best separated into phases, sub-blocks, and instructions. Therefore, using the Method Wizard to build methods for different applications is recommended.

Create a new method

To create a new method using the ***Method Wizard*** in the ***Method Editor***, use the following steps:

Step	Action
1	Click the ***Create a new method*** icon in the ***UNICORN Method Editor***. 

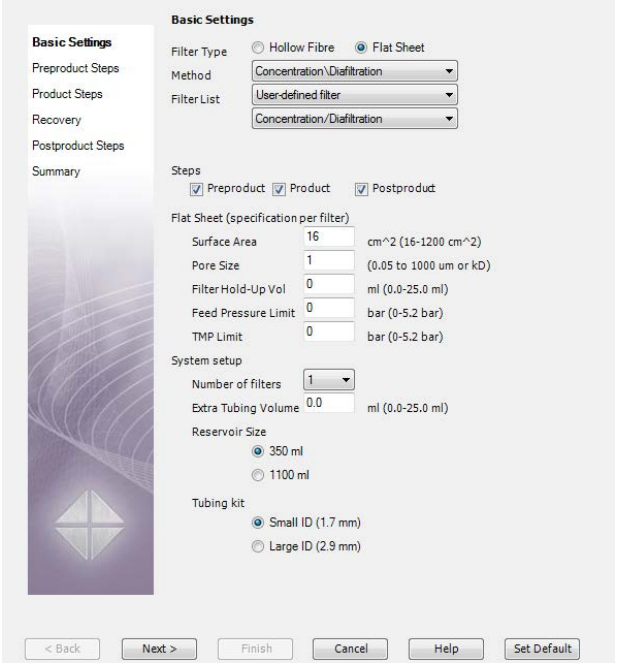
Step	Action
2	Select the ÄKTAcrossflow system, choose ***Method Wizard,*** and click ***OK***.



Result:

A dialog of ***Basic Settings*** will be displayed.

Step **Action**

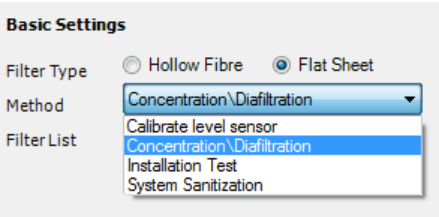

Basic Settings
Preproduct Steps
Product Steps
Recovery
Postproduct Steps
Summary
Basic Settings
Filter Type
Hollow Fibre
Flat Sheet
Method
Concentration\Diafiltration
Filter List
User-defined filter
Concentration/Diafiltration
Steps
Preproduct
Product
Postproduct
Flat Sheet (specification per filter)
Surface Area
16
cm^2 (16-1200 cm^2)
Pore Size
1
(0.05 to 1000 um or kD)
Filter Hold-Up Vol
0
ml (0.0-25.0 ml)
Feed Pressure Limit
0
bar (0-5.2 bar)
TMP Limit
0
bar (0-5.2 bar)
System setup
Number of filters
1
Extra Tubing Volume
0.0
ml (0.0-25.0 ml)
Reservoir Size
350 ml
1100 ml
Tubing kit
Small ID (1.7 mm)
Large ID (2.9 mm)
< Back
Next >
Finish
Cancel
Help
Set Default

Step	Action
3	To obtain default values in the ***Method Wizard***:

a. Click ***Set Default***. This is only possible in the first dialog.

b. Choose between two filter types, either ***Hollow Fibre*** or ***Flat Sheet***.

c. In the ***Method*** list, the type of process is selected



Note:

The system defaults to a ***Concentration/Diafiltration*** *method, but other methods are also available:*

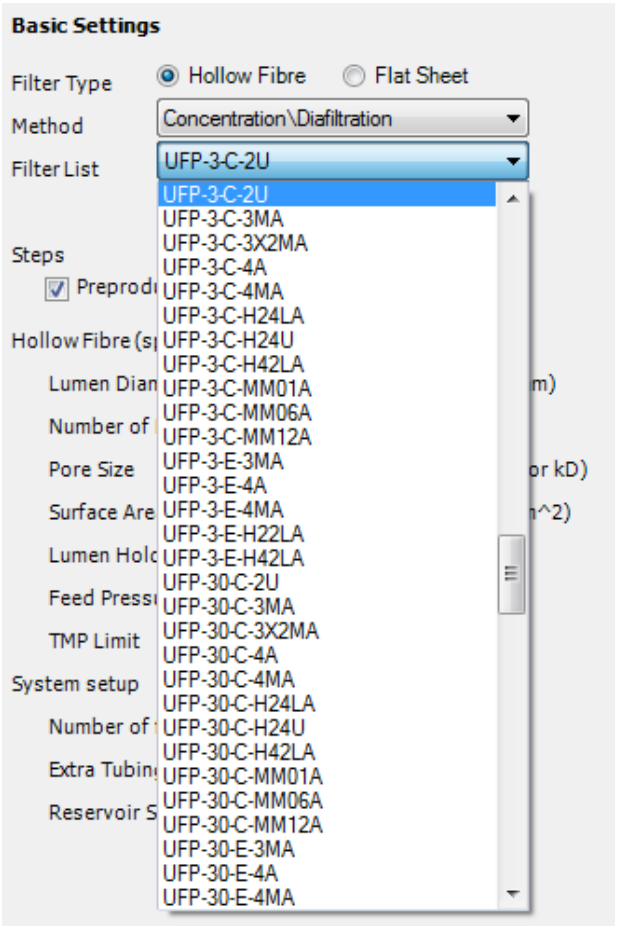
- ***Calibrate level sensor***
- ***Installation Test***
- ***System Sanitization***

These methods are discussed in *Section 3.6 Calibrate the level sensor, on page 46*, *Section 11.1 System sanitization, on page 218*, and the *ÄKTAcrossflow Installation Test Guide (11001235)*.

Step	Action
4	If ***Filter Type*** →***Hollow Fibre*** has been selected, the ***Filter List*** will display available GE hollow fibre filters.

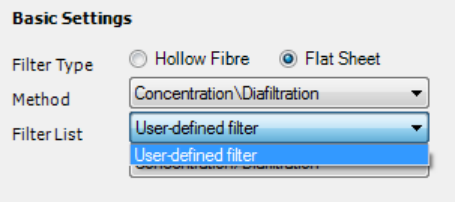
a. ***Concentration/Diafiltration*** creates a standard ultrafiltration method.

b. ***UF Process Optimization*** creates a TMP and crossflow rate scouting method. This process is discussed further in *Chapter 9 Process optimization in Ultrafiltration, on page 202*.

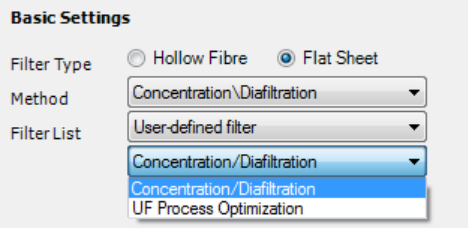


If ***Filter Type*** →***Flat sheet*** has been selected, the filter type ***User-defined filter*** is displayed in the ***Filter List*** window.

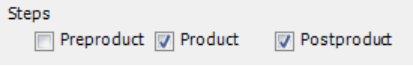
Step	Action



An additional choice of method type is available for flat sheet cassettes:



5 Below the ***Filter List***, select the type of steps (***Preproduct***, ***Product***, and/or ***Postproduct***) to include in the method.



6 If ***Filter Type*** →***Hollow Fibre*** has been selected, the specifications are pre-populted. These can be edited by the user.

If ***Filter Type*** →***Flat sheet*** has been selected, the user must fill in the specification values for:

a. ***Surface area***

b. ***Pore size***

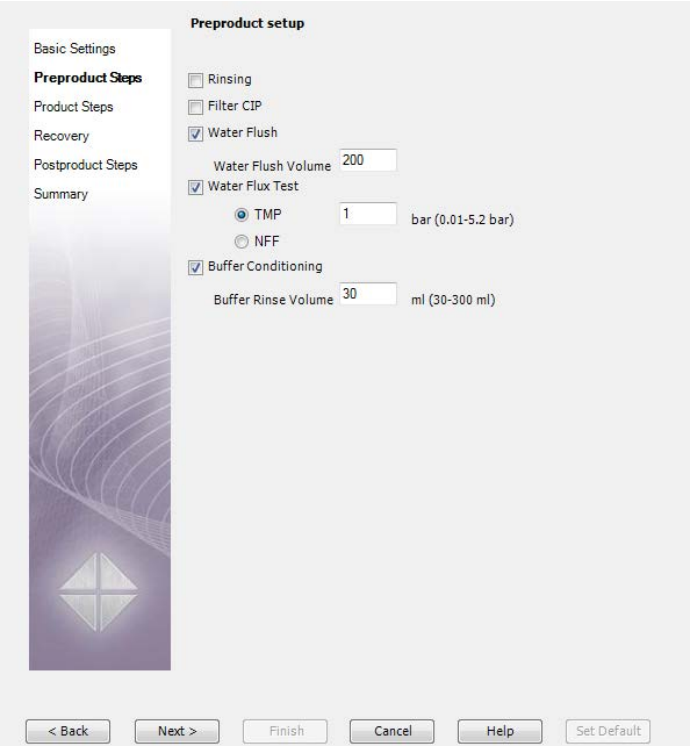
c. ***Filter hold-up volume***

d. ***Feed pressure limit***

e. ***TMP limit***

Step	Action
7	In the ***System setup*** section: **a.** Select the number of filters (only necessary when using several filters assembled together in parallel). **b.** Input any extra tubing volume used in the recirculation loop (e.g., when connecting in a separate filter holder). **c.** Select the reservoir size (350 mL or 1100 mL) and tubing kit (large i.d. or small i.d.) used in the recirculation loop. The inputs for filter hold-up volume, tubing i.d., and extra tubing volume are used by the system to calculate the recirculation hold-up volume. To get help instructions for each ***Method Wizard*** dialog, click ***Help*** or press the F1 key. To go back to the previous dialog, click ***Back***. To stop the ***Method Wizard***, click ***Cancel***. To proceed with the next dialog, click ***Next***.

Step	Action
8	In each dialog, select the appropriate parameter values and click ***Next*** to continue. Continue with inputs on all dialog pages.



Finalize the method

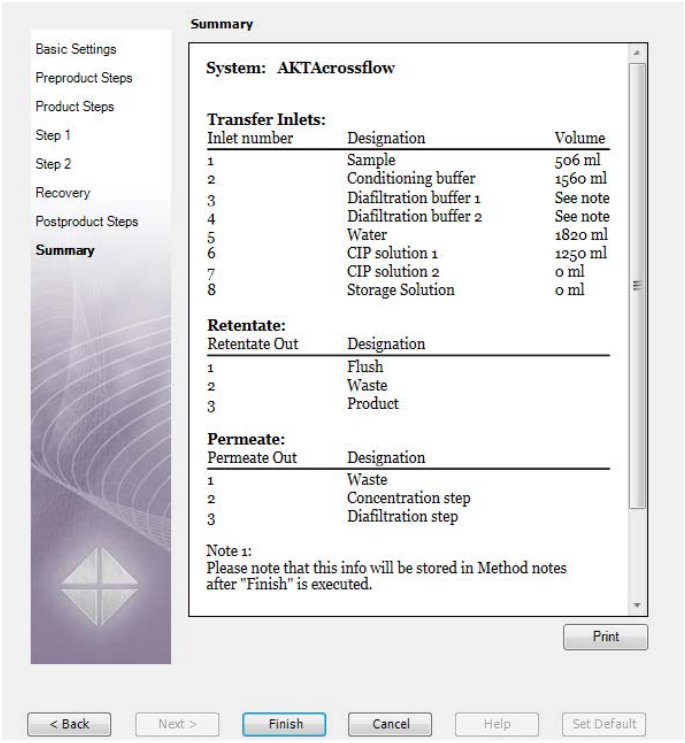
After a series of dialogs (depending on the choice of preproduct, product, and post-product), a ***Summary*** dialog is shown.

A list of calculated volumes of required solutions is displayed.

The sample volume includes 6 mL extra for priming, which will go to waste.

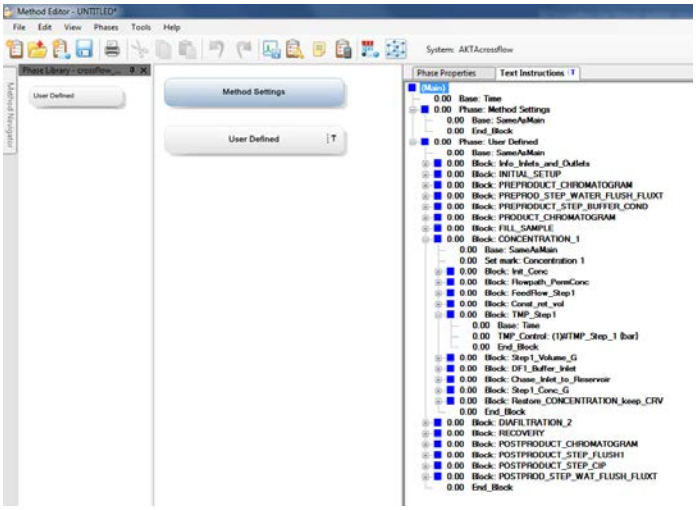
The diafiltration buffer volumes are not given; in cases where the sample load is terminated by the air sensor, it is not possible to estimate these volumes. The end user should calculate the required volumes and ensure that there is enough diafiltration buffer to meet this requirement.

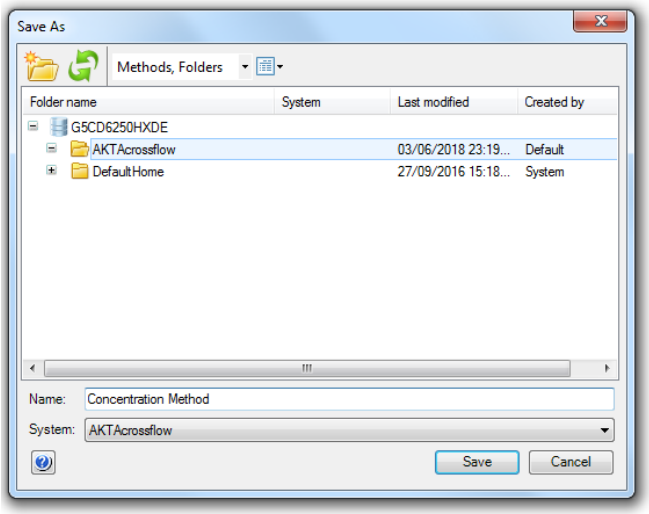
Note: *To prepare a run with correct solutions, it is recommended to print this list using the **Print** button. This list will also be displayed in **Method Notes**.*



To finish and save the method, use the following steps:

Step	Action
1	Click ***Finish*** in the ***Summary*** dialog to finalize the creation of the new method. UNICORN will create a complete method, with each step contained with a sub-block of a ***User Defined*** phase.

Step	Action
2	To display the text instructions of the created method, click the ***Text Instructions*** tab. 
3	To view and expand the method, click on any plus sign. ***Note:*** *The method can be edited in the **Text Editor** as described in Section 4.2.2 Create crossflow methods using Text Instructions, on page 73.*
4	To save the method, select ***File*** →***Save***.
5	Select save location and enter a method name.
6	Select ***System***.

Step	Action
7	 Click ***OK***.

4.2.2 Create crossflow methods using Text Instructions

The ***Text Instructions*** editor in the ***Method Editor*** can be used to build methods step by step. The editor can also be used to modify instructions in methods created by the ***Method Wizard***.

The first line of the empty method contains the main ***Base***. The base provides a way to progress to new instructions via breakpoints. Individual instructions can be added into the method, but can only be added from inside a ***Phase***. Methods are built up using phases, where each phase corresponds to a step in a filtration run. See *UNICORN Method Manual* for more information about method structure, definitions, and concepts of methods in UNICORN.

The principle of inserting an instruction in a method is as follows:

1. Enter the ***Breakpoint*** in minutes or volume (depending on base) for the instruction.
2. Select an individual instruction under the ***Instructions*** group.
3. Select the macro instruction in the dropdown list or enter the parameter.
4. Click ***Insert*** to add the instruction to the method.

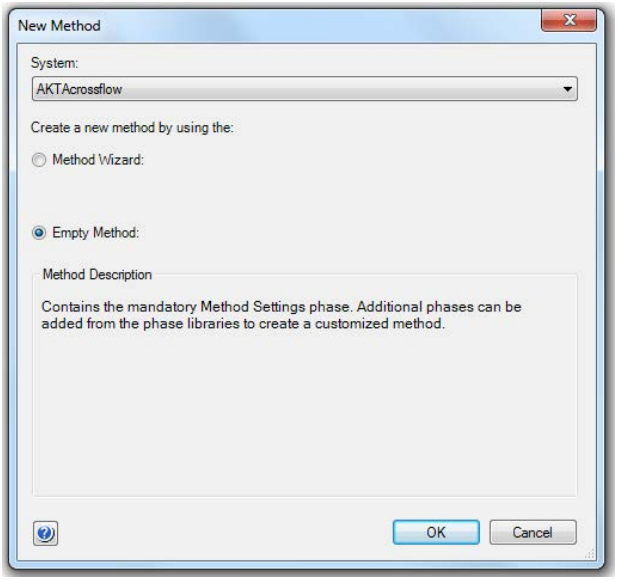
Note: *All Strategy instructions for the ÄKTAcrossflow are listed in Chapter 14 Strategy instructions, on page 268.*

Create a new method

Use Text Instructions to create a method as follows:

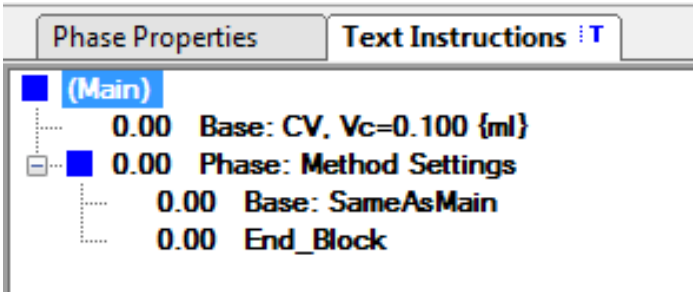
Step	Action
1	Select the ***Method Editor*** module in UNICORN.
2	To create the new method. click on the ***Create a new method*** icon in the UNICORN ***Method Editor***. 

Step	Action
3	Select the ÄKTAcrossflow system, choose ***Empty Method***, and click ***OK***.

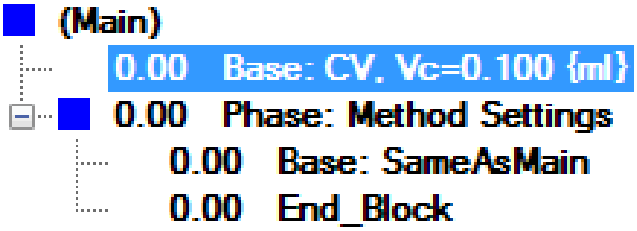
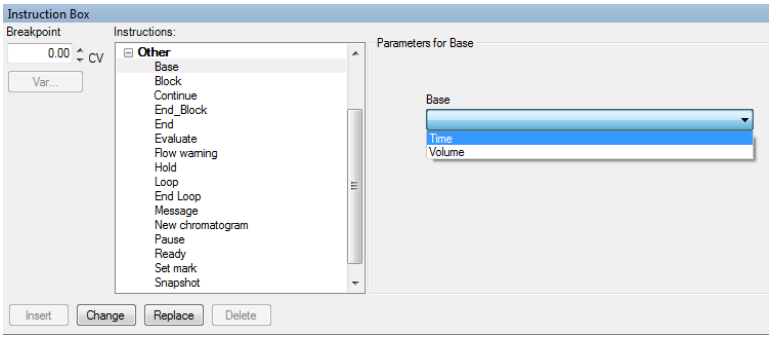


Result:

UNICORNwill create an empty method with a main base and mandatory ***Method Settings*** phase.



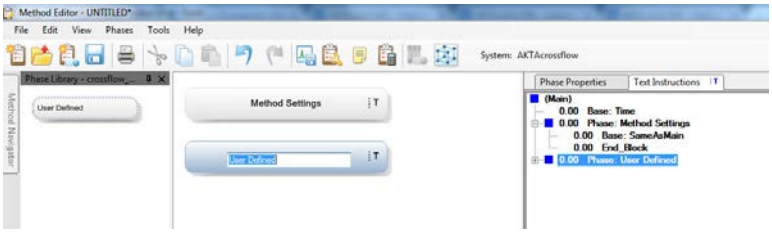
4	Select the ***Text instructions*** tab to start building up the method.

Step	Action
5	Select the parameters for the base. Highlight the Main Base instruction row.  UNICORN will automatically select the Instruction group ***Other*** and instruction ***Base*** in the ***Instruction Box***.
6	From the drop-down menu, select the base type. For ÄKTAcrossflow, the first base parameter can be either: **a.** Time *or* **b.** Volume ***Note:*** *By default in UNICORN,* ***Column Volume (CV)*** *is set as the base. This is not useful in ÄKTAcrossflow methods and will lead to incorrect methods.*  Click ***Change***.

Add instructions to a method

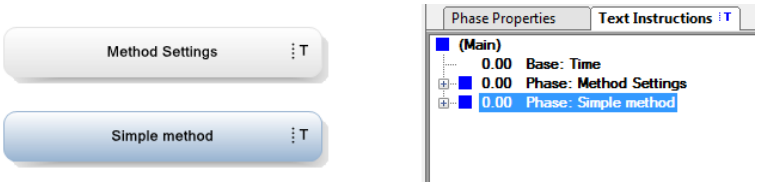
To add instructions to the method, create a new phase using the following steps:

Step	Action
1	Drag the ***User Defined*** phase in the ***Predefined Phases Library*** into the ***Method Outline*** pane. Rename the phase if desired.



Result:

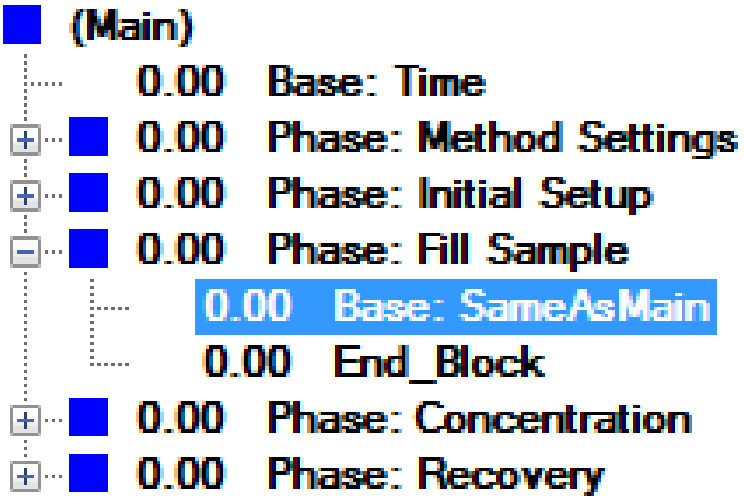
UNICORNinserts a new, empty phase into the method.



Note:

A separate base can be set for every phase. By default it will be set as ***Same As Main****, which refers to the base set in the first line of the method.*

Step	Action
2	To insert instructions into the phase, highlight ***Base*** →***SameAsMain*** and select instructions from the ***Instruction Box*** at the bottom of the ***Method Editor*** page.

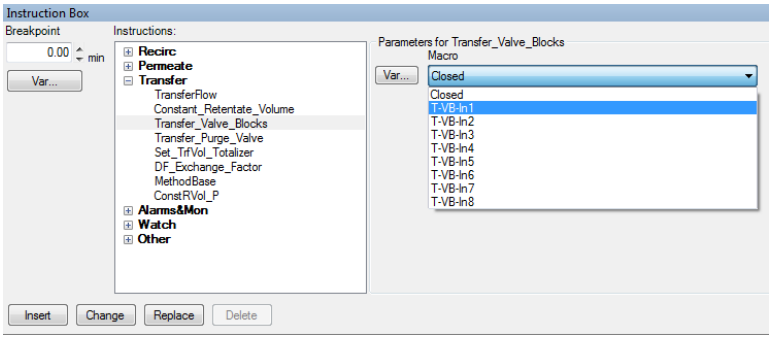


3	Select the group of instructions, for example ***Transfer***.

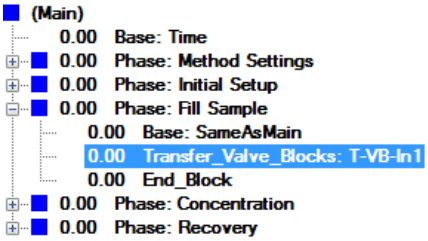
Note:

Instructions are grouped according to functionality: for example, ***Recirc*** *contains all of the relevant instructions for the recirculation pathway;* ***Permeate*** *contains the instructions to start the filtration process and control the permeate pathway; and* ***Transfer*** *contains the instructions to bring liquid into the reservoir. (See Chapter 14 Strategy instructions, on page 268 for a description of the instructions).*

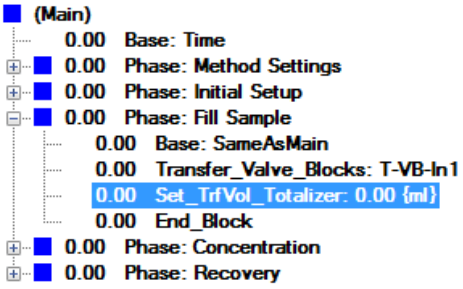
Step	Action
4	Select an instruction within the group.



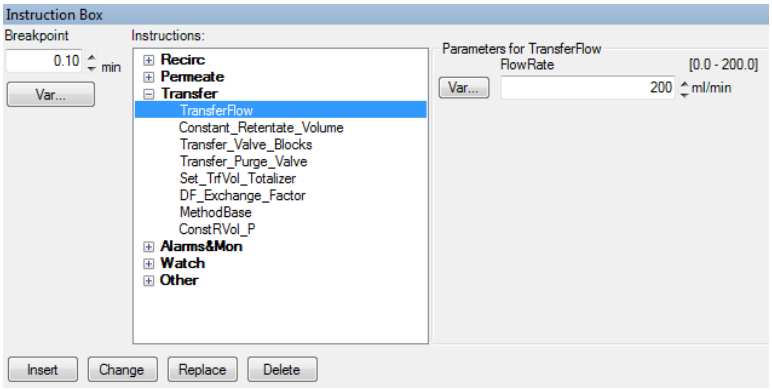
5	Enter a parameter value or select valve position from the dropdown menu.
6	Click ***Insert*** to place the instruction in the method.

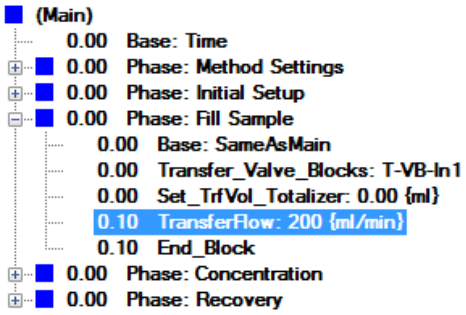


7	To add second instruction, highlight the first instruction.
8	Select the desired instruction in the ***Text Instructions*** box

Step	Action
9	click ***Insert***.*Result:*The new instruction will be added below the highlighted instruction. *Result:* click ***Insert***.The new instruction will be added below the highlighted instruction. 
10	To delete an instruction row, select the row and click ***Delete***.

Step	Action
11	By default, both instructions are executed by UNICORN at breakpoint 0 minutes. To introduce a delay in the execution of instructions, change the breakpoint for the next instruction, and click ***Insert***.

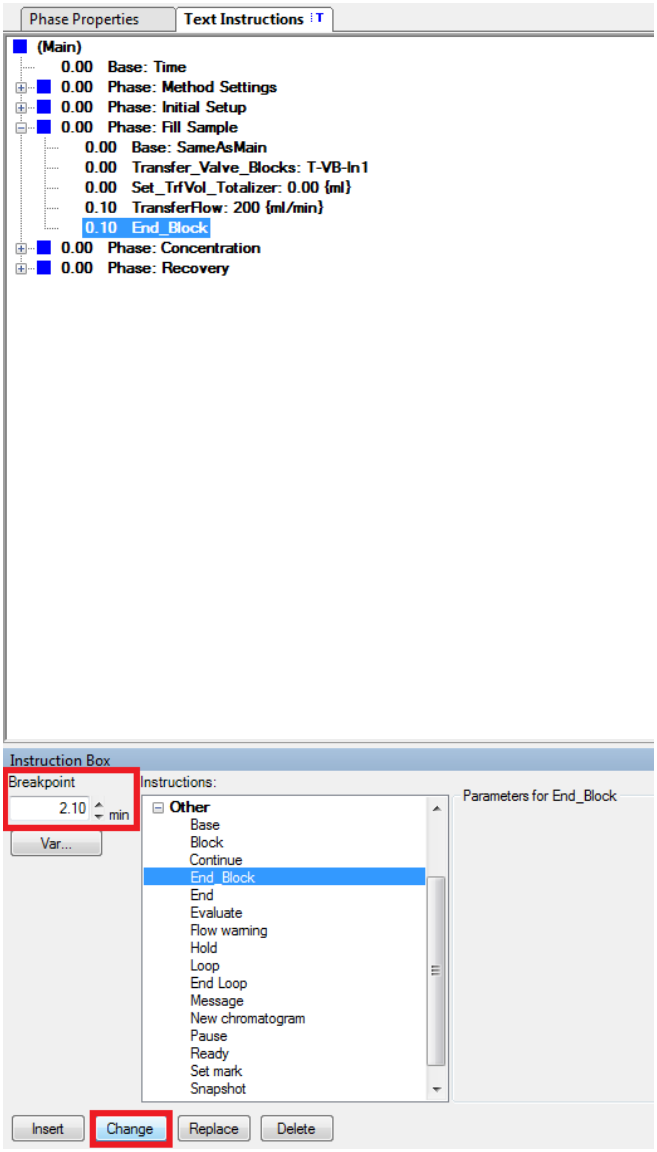




Note:

The instruction to end the phase, ***End_Block****, automatically adjusts to the new breakpoint. This instruction can be changed to further introduce delay into the method. Highlight* ***End_Block*** *and in the* ***Instruction Box****, change the breakpoint to 2 minutes, and click* ***Change****.*

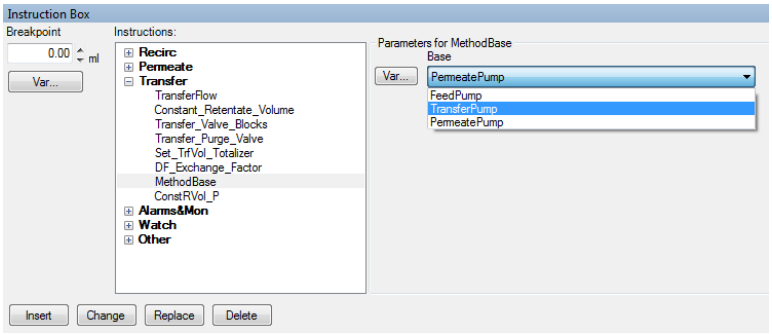
Step	Action


Phase Properties
Text Instructions
(Main)
0.00 Base: Time
0.00 Phase: Method Settings
0.00 Phase: Initial Setup
0.00 Phase: Fill Sample
0.00 Base: SameAsMain
0.00 Transfer_Valve_Blocks: T-VB-In1
0.00 Set_TrfVol_Totalizer: 0.00 {ml}
0.10 TransferFlow: 200 {ml/min}
0.10 End_Block
0.00 Phase: Concentration
0.00 Phase: Recovery
Instruction Box
Breakpoint
2.10
min
Var...
Instructions:
Other
Base
Block
Continue
End_Block
End
Evaluate
Flow warning
Hold
Loop
End Loop
Message
New chromatogram
Pause
Ready
Set mark
Snapshot
Parameters for End_Block
Insert
Change
Replace
Delete

Step	Action

Note:

If the base in the phase is time, all breakpoints are given in time. If volume is selected as base, the breakpoints will be given in volume.



12 Repeat steps 1 to 5 until all instructions are inserted in the method.

13 To change a breakpoint value or parameter in an instruction, highlight the instruction, enter a new value, and click ***Change*** or ***Replace***.

Note:

When changing parameters, the instructions both work in the same way. However, when changing breakpoints, ***Change*** *will recalculate all following breakpoints to maintain the length of time or volume between instructions, whereas* ***Replace*** *will not recalculate the breakpoints and will only change the breakpoint for the highlighted instruction.*

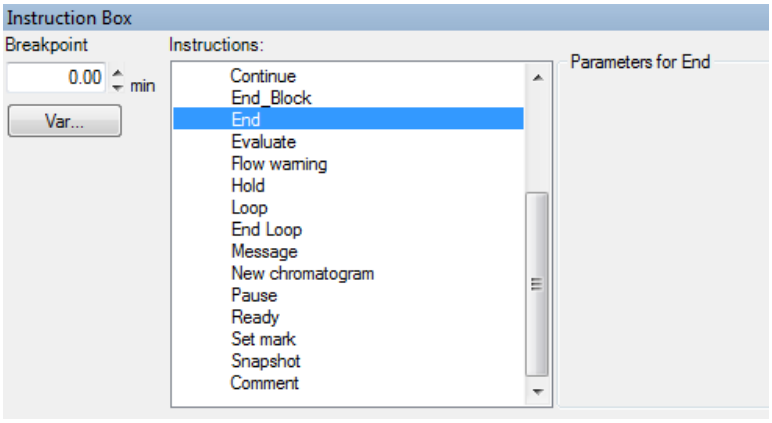
Tip:

To create separate steps within the method, create new phases for each step.

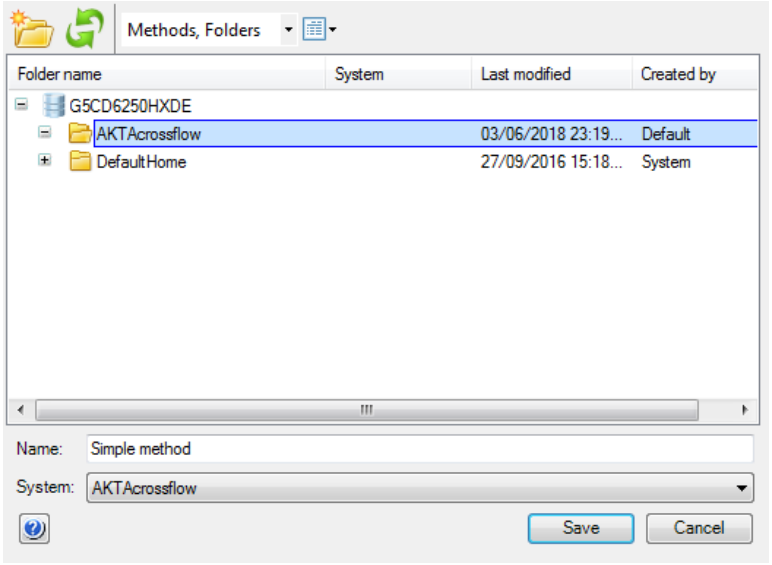
Finalize the method

Finalize and save the new method as follows:

Step	Action
1	To end the method, select Instruction group ***Other*** and the instruction ***End***. UNICORN will also automatically end when no other instructions are present to execute.



2	To save the method, select ***File*** →***Save***.



3	Choose folder location, and select system.

Step	Action
4	Enter a method name and click ***Save***.

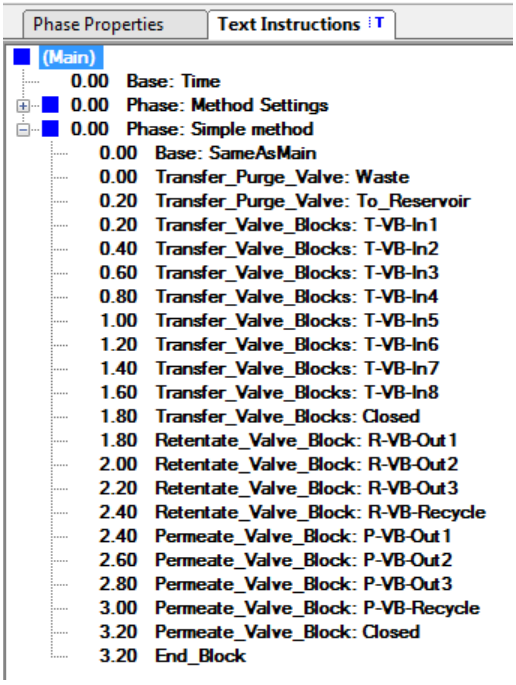


Figure 4.1: Example of a simple ÄKTAcrossflow method

5 Perform crossflow runs manually

About this chapter

This chapter contains information on how to perform and monitor ÄKTAcrossflow methods manually without using the ***Method Wizard***.

In this chapter

Introduction

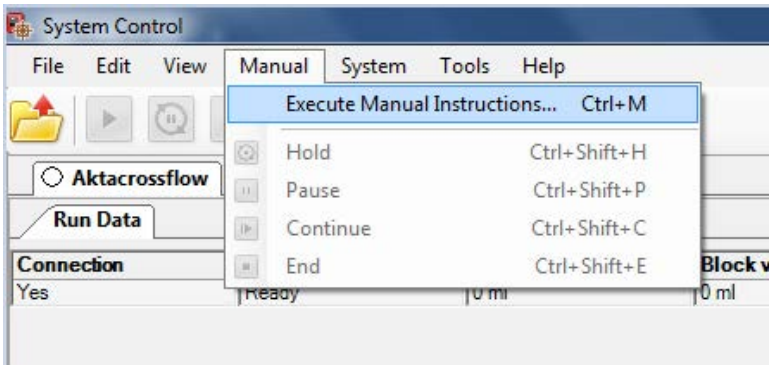
The most convenient way to perform crossflow runs is to use the ***Method Wizard*** to create methods. However, it is always possible to perform process steps using the manual mode in UNICORN via the ***System Control*** module.

Note: *All Strategy text instructions and parameters are listed in Chapter 14 Strategy instructions, on page 268.*

5.1 Executing text instructions

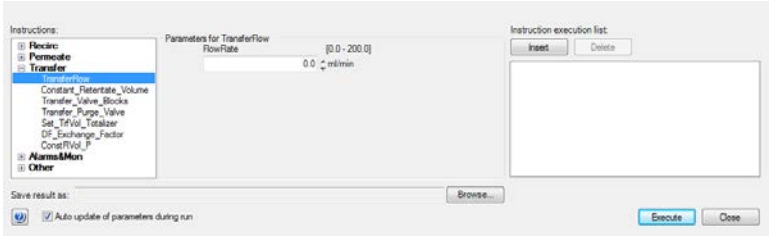
To manually execute text instructions, use the following procedure.

Step	Action
1	In the ***System Control*** window in UNICORN, select ***Manual*** →***Execute Manual instructions***.



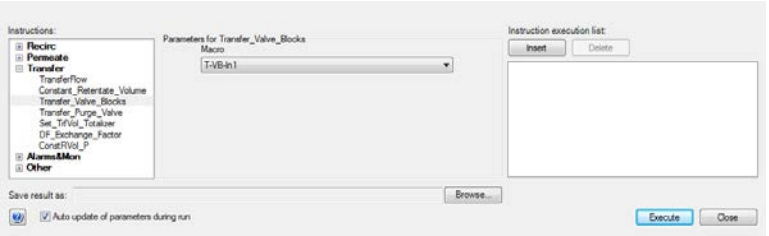
Result:

The ***Manual instructions*** dialog is displayed.

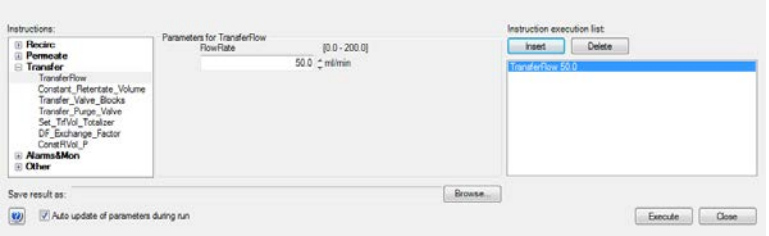


2	Select instruction group, for example, ***Transfer***.
3	Select instruction, for example, ***Transfer_Valve_Blocks***.
4	Select an inlet valve position from the drop-down menu.

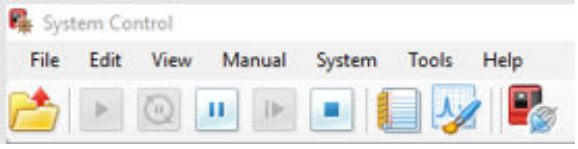
Step	Action
5	Click ***Execute***.



6	Select next instruction, for example ***TransferFlow***.



7	Enter a parameter value, for example 50 mL/min.
8	Click ***Execute***. ***Note:*** *It is also possible to* ***Insert*** *a series of instructions into the* ***Instruction execution list*** *and click* ***Execute*** *so that UNICORN executes all instructions at once. There are, however, some limitations in executing valve and pump instructions simultaneously on the ÄKTAcrossflow; therefore, it is recommended to execute each instruction individually.*
9	To stop the operation, click the ***End*** or ***Pause*** button in the ***System Control*** window.



Safety precautions

General

WARNING

Do not operate the ÄKTAcrossflow system at pressures above the specified maximum pressure (5.2 bar).

WARNING

Always use appropriate Personal Protective Equipment (PPE) during operation and maintenance of this product.

CIP method

Be very careful when running a method using a CIP (clean-in-place) solution containing sodium hydroxide (NaOH).

WARNING

NaOH is corrosive and therefore dangerous to health. Avoid spillage and wear safety glasses, safety gloves, and protective lab coat.

CAUTION

Always make sure that the filters and system components are compatible with sodium hydroxide at the concentration, time, and temperature used.

5.2 Monitoring the run

Introduction

This section describes the data shown in ***System Control*** during a run and the procedure to customize the view of the different panes. It also describes how to enable alarm and error notifications. It is possible to view the ongoing method run in the ***System Control*** module.

The current system state is shown in the ***System state*** box in the ***Run Data*** pane. For example, it may state ***Method Run***, ***Manual Run***, or ***Hold***.

Selected curves are shown in the ***Chart*** pane.

The current flow path is shown in the ***Process Picture*** pane.

Note: *To find an overview of the* ***System Control*** *user interface, see UNICORN 7 System Control Manual.*

Open the Customize dialog

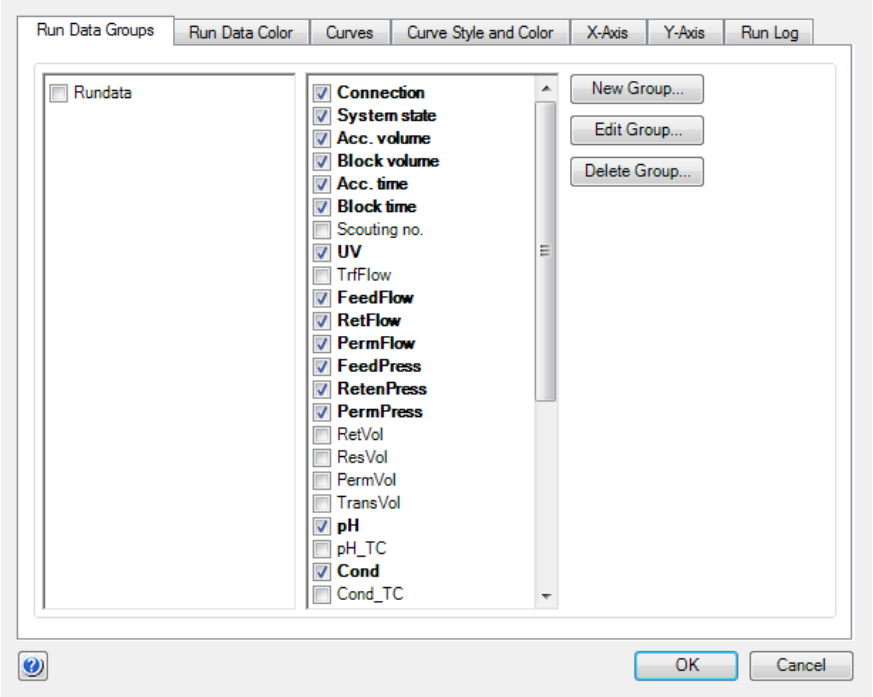
To customize displayed information and data in the different panes, use one of the following options.

1. *Either* click the ***Customize*** button in the tool bar 

   or

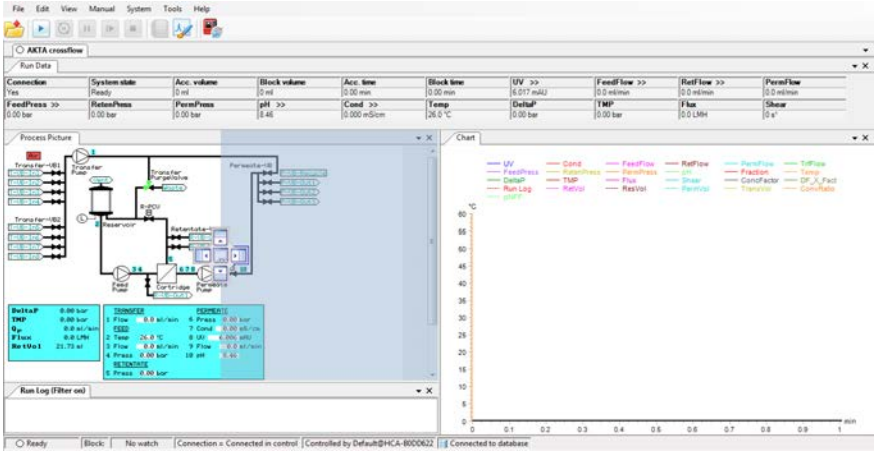
2. Right click in the different panes (except ***Process Picture***) and click ***Customize***.

*Result:*The ***Customize*** dialog opens.

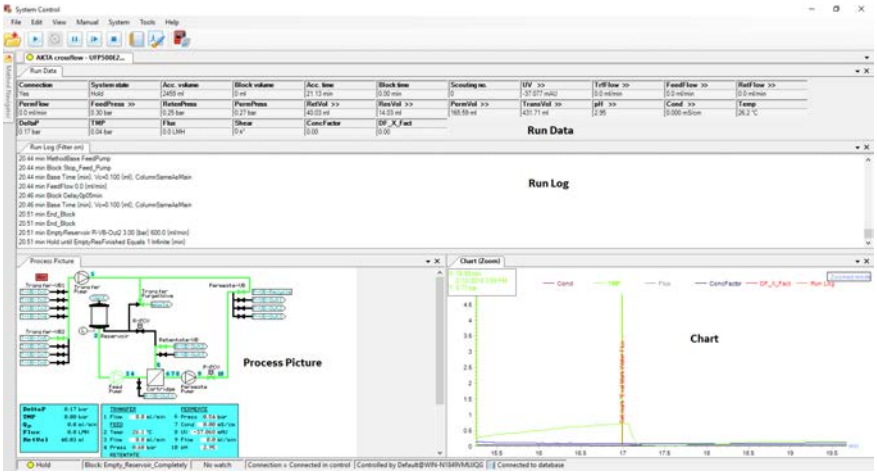


Note: *Further information about the settings in the **Customize** dialog can be found in the **Online Help** and in the UNICORN System Control manual.*

To rearrange the display panes, drag and drop them to the desired location.



Example of displayed information:


Run Data
Run Log
Process Picture
Chart

6 Create preproduct steps using the Method Wizard

About this chapter

This chapter provides information on how to create preproduct steps using the ***Method Wizard***. Information is also provided about how to create preproduct methods for Ultrafiltration and Microfiltration using the ***Method Wizard***.

In this chapter

6.1 Preproduct steps: Introduction

The ÄKTAcrossflow ***Method Wizard*** can create a series of steps to make sure that a filter is in the proper condition before performing a process run. These steps include:

- ***Rinsing***
- ***Filter CIP (Clean-in-Place)***
- ***Water Flush***
- ***Water Flux Test*** (also called Normalized Water Permeability, or NWP)
- ***Buffer Conditioning***

Different combinations of the steps above may be used.

If the system has not been sanitized recently, it may be necessary to start with a sanitization of the system (see *Section 11.1 System sanitization, on page 218*). A sanitization of the system is also recommended when introducing new products (proteins, viruses, or cells) to the system, to avoid cross-contamination.

The following table summarizes the recommended preproduct steps.

Condition	Rinsing	Filter CIP 1	Water flush	Filter CIP 2	Water flush	Water flux test	Buffer conditioning
New filter	×					×	×
Same filter, after recommended postproduct procedure with terminal water flush and flux test, no storage							×
Different filter, after recommended postproduct procedure plus storage					×	×	×

6.2 Preproduct steps: Description

Rinsing

New ultrafilters are typically delivered in a storage solution such as glycerol, which helps to prevent the filter from drying out. Microfilters are often delivered dry. Both types of filters should be rinsed with water prior to use.

This step will:

- Prime the transfer inlet valve position 5 (**T-VB-In5**) tubing to waste,
- Add a small volume of water to the reservoir to rinse the retentate loop,
- Empty the reservoir,
- Refill the reservoir to a small volume and flush 2 mL water per cm^2 surface area of the filter out through permeate valve block position 1 (**P-VB-Out1**),
- Empty the reservoir.

Note: *Some hollow fibers do not wet out very well with water. If problems occur, for example, if you experience a failed water flux test, it might be necessary to flush the filter manually with an alcohol solution, such as 20% to 30% isopropanol or 20% to 30% ethanol. For more information, refer to the Hollow fiber operating guide.*

Filter CIP

A new filter does not necessarily need to be sanitized with a CIP step. However, if a filter is re-used, depending on the postproduct processing, a preproduct CIP step may be desirable.

The filter CIP preproduct step includes an option to perform two filter CIP procedures with an optional water flush between.

This step will:

- Prime the CIP transfer inlet valve positions tubing to waste,

  Note: *If CIP 1 only is chosen, only transfer valve block 6 (**T-VB-In6**) is primed; if CIP 2 only is chosen, only transfer valve block 7 (**T-VB-In7**) is primed. If both CIP 1 and 2 are selected, both inlets will be primed.*

- Add CIP solution to the reservoir to rinse the retentate loop,
- Empty the reservoir,
- Either fill the reservoir to the maximum volume (small reservoir) or fill to a specified fill volume (large reservoir),
- Rinse 30 mL CIP solution to waste through **P-VB-Out1**,

- Recirculate the permeate back into the reservoir for the ***Length of Time*** specified in the dialog,
- Empty the reservoir.

The process is repeated if CIP 2 is chosen, with CIP 2 solution on **T-VB-In7**.

If a water flush is chosen between CIP 1 and 2, the system will:

- Prime the transfer inlet valve position 5 (**T-VB-In5**) tubing to waste,
- Add a small volume of water to the reservoir to rinse the retentate loop,
- Empty the reservoir,
- Refill the reservoir to a small volume and flush 10 mL water out through permeate valve block position recycle (**P-VB-Recycle**) to waste (**Transfer_Purge_Valve** to **Waste**),
- Empty the reservoir.
- ***Note:*** *The system will not be sanitized. For sanitization of the system, see Section 11.1 System sanitization, on page 218*

Water flush

A water flush step is identical to the rinse step, unless a CIP step has been selected. If CIP is chosen, the water flush step will fill the reservoir to the maximum volume (350 mL for the small reservoir and 1100 mL for the large reservoir) and empty, to make sure that the CIP solution has been removed. It is always recommended to select a water flush after a filter CIP step where NaOH was used. This step should also be used if a filter has not been rinsed with water before a water flux test is performed.

With no preproduct CIP, this step will:

- Prime the transfer inlet valve position 5 (**T-VB-In5**) tubing to waste,
- Add a small volume of water to the reservoir to rinse the retentate loop,
- Empty the reservoir,
- Refill the reservoir to a small volume and flush 2 mL water per cm^2 surface area of the filter out through permeate valve block position 1 (**P-VB-Out1**),
- Empty the reservoir.

If following a preproduct CIP step, this step will:

- Prime the transfer inlet valve position 5 (**T-VB-In5**) tubing to waste,
- Fill the reservoir to maximum volume (350 mL for the small reservoir and 1100 mL for the large reservoir) and empty,
- Add a small volume of water to the reservoir to rinse the retentate loop twice,
- Empty the reservoir,

- Refill the reservoir to a small volume and flush 2 mL water per cm^2 surface area of the filter out through permeate valve block position 1 (**P-VB-Out1**),
- Empty the reservoir.

Water flux test

A water flux test measures the water permeability of a filter, to control the quality status of the filter. A permeate flux value is often normalized to 1 bar transmembrane pressure and corrected to a temperature of 25°C, and is then called the normalized water permeability (NWP; also called normalized water flux, or NWF). By comparing obtained water fluxes as a function of usage over time, it is possible to determine the cleaning efficiency of the filter CIP and lifetime of a filter.

Note: *Always perform a water flush or rinse before a water flux test, to make sure that the filter is thoroughly flushed with water.*

It is recommended to perform the test before a product step and after a product step and filter cleaning.

The filtration control mode is dependent on the filter type used.

For flat sheet cassettes, TMP control mode is used. The default TMP value is 1 bar, but this can be edited by the user.

For ultrafilter hollow fibers, TMP control mode is used. The default TMP value is 1 bar, but this can be edited by the user. It is recommended to set the TMP value to 0.5 bar or lower for high molecular weight hollow fiber ultrafilters (≤ 500 kD).

For microfilter hollow fibers (cut off larger than 0.1 µm), ***Normal Flow Filtration*** mode is used. ***Feed flow*** or ***Feed pressure*** can be selected as a feed control and a value is entered.

This step will:

- Prime the transfer inlet valve position 5 (**T-VB-In5**) tubing to waste,
- Add a small volume of water to the reservoir to rinse the retentate loop,
- Empty the reservoir,
- Fill a small volume of water to the reservoir,
- Set the system to total recycle (**P-VB-Recycle**, **Transfer_Purge_Valve** to **Reservoir**),
- Set the filtration control mode and wait until the flux has stabilized,
- Measure the permeate flux and set a ***Set_Eval_Mark*** with the parameter ***Normalized_Water_Flux*** for easy analysis in the ***Evaluation*** module.

Data from water flux testing can be analyzed in the ***Evaluation*** module of UNICORN. In *Figure 6.1, on page 97*, normalized water flux results from a series of measurements are plotted against the number of performed runs with a filter. A standardized temperature correction table compensates for temperature effects due to viscosity. Results are compared to previous tests and provide information about the quality status of the filter.

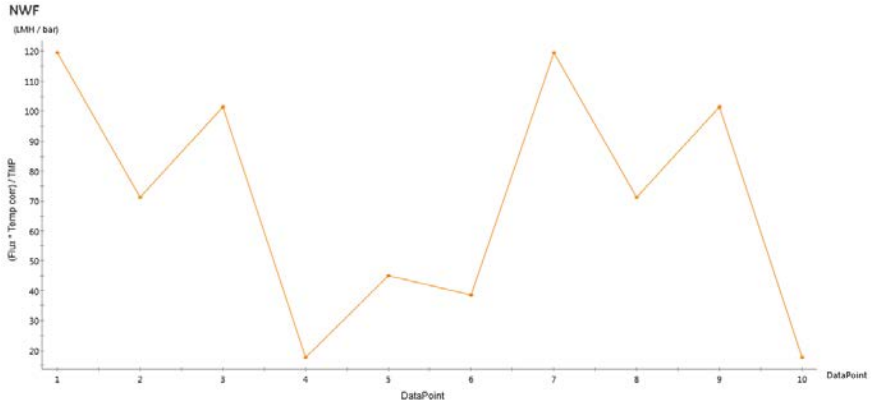


Figure 6.1: Example of plotted normalized water flux values

Note: *Some hollow fibers do not wet out very well with water. If problems occur, for example, if the water flux test fails, it may be necessary to flush the filter manually with an alcohol solution, such as 20% to 30% isopropanol or 20% to 30% ethanol. For more information, refer to the Hollow Fiber Operating Guide.*

Note: *When comparing status of a filter as a function of time and number of uses, use the same filtration mode parameter each time the water flux test is run.*

Buffer conditioning

The buffer conditioning step replaces the liquid in the filter with a buffer that is suitable a processing step with product. The buffer conditioning step will:

- Prime the transfer inlet valve position 2 (**T-VB-In2**) tubing to waste,
- Add a small volume of buffer to the reservoir to rinse the retentate loop,
- Empty the reservoir,
- Fill a small volume of buffer to the reservoir,
- Flush the filter with the selected volume, through permeate recycle to waste (**P-VB-Recycle**, ***Transfer_Purge_Valve*** to ***Waste***),
- Set the system to total recycle (**P-VB-Recycle**, ***Transfer_Purge_Valve*** to ***Reservoir***) for 5 minutes,

- Empty the reservoir.

Note: *If a CIP has been performed, we recommend performing a buffer conditioning after the water flush to ensure that the pH in the system is suitable for any following product steps.*

6.3 Preproduct steps: Method Wizard dialogs

About this section

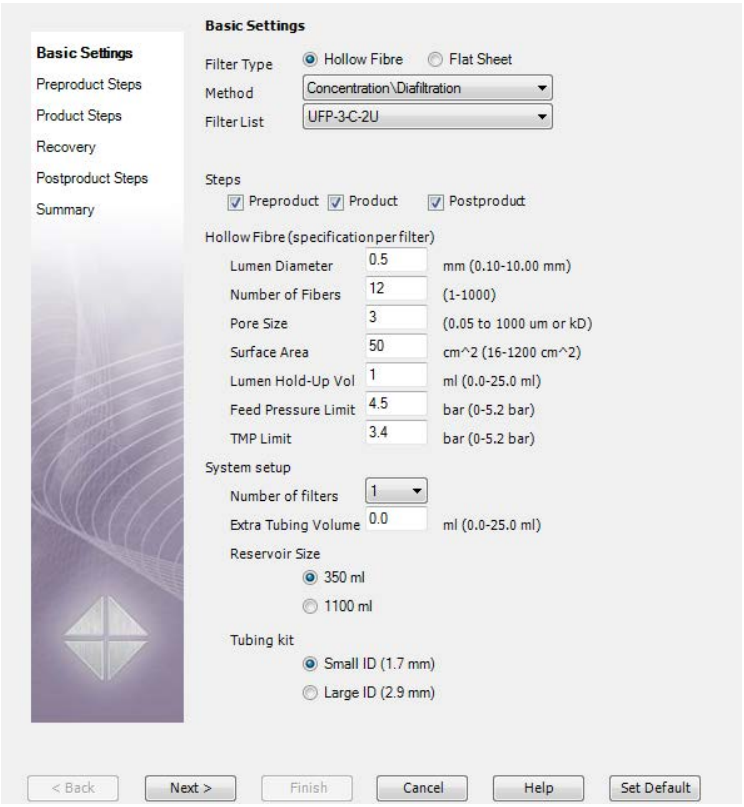
This section provides information on how to create preproduct steps in the ***Method Wizard*** when using hollow fibres and flat sheet cassettes, and provides a description of each step.

In this section

6.3.1 Basic settings dialog

Basics settings dialog overview

The image below shows an example of the ***Basic Settings*** dialog.

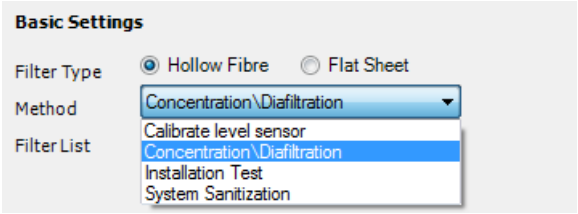


Basic Settings →Hollow Fibre

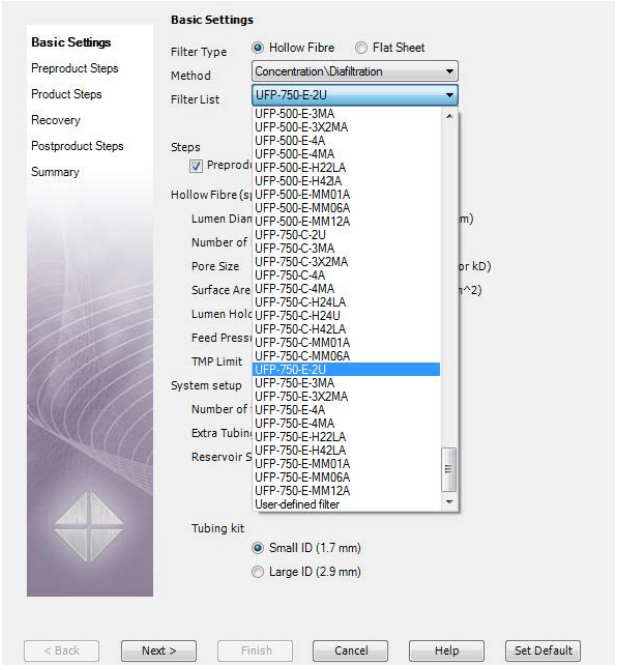
To enter the basic settings for use with hollow fibre filters, use the following procedure:

Step	Action
1	In ***Basic Settings***, select ***Hollow Fibre***.

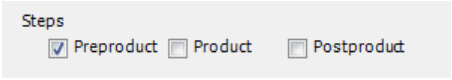
Step	Action
2	In the ***Method*** list, select ***Concentration/Diafiltration***.



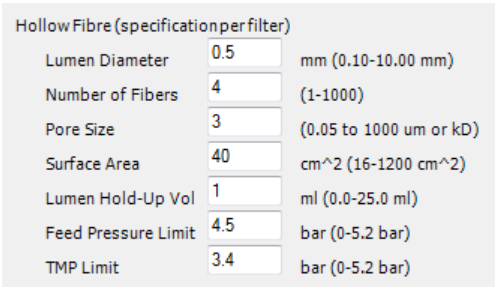
3	In the ***Filter List***, compatible GE hollow fiber filters are displayed. Select the desired filter, or if using a hollow fiber from another manufacturer, select ***User-defined*** filter.



4	Select ***Preproduct*** process step.



Step	Action
5	The ***Hollow Fibre (specification per filter)*** area displays the recommended default values for the selected filter. The values can be edited as desired.



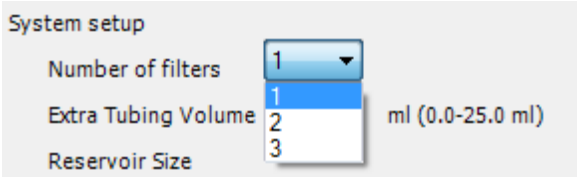
Note:

*If **User-defined** filter has been selected, enter the lumen diameter, number of fibers, surface area, lumen hold-up volume, feed pressure limit, and TMP limit. This information is usually available from the manufacturer.*

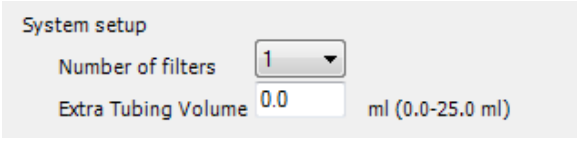
Note:

When using TMP as the filtration control mode in a method, always choose a TMP value in the product steps that is below the TMP limit. Otherwise, the TMP limit may lead to a TMP pressure alarm, which will pause the run.

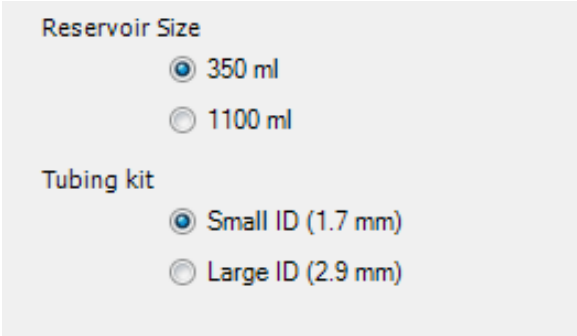
6	If several filters are assembled together in parallel, select in the ***System setup*** section the number of filters (1 to 3). If only one filter is used, keep the default value of 1.



7	If any extra tubing is used on the recirculation loop, calculate the extra volume added and enter the value in the ***Extra Tubing Volume*** box.



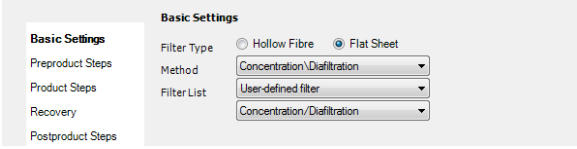
Step	Action
8	Select the reservoir size and tubing kit i.d. used in the recirculation loop.



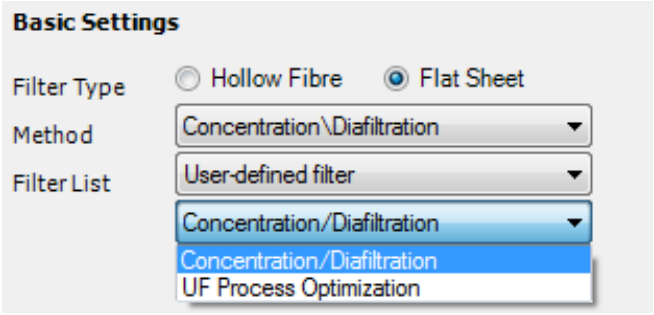
Basic Settings →Flat Sheet

To enter the basic settings for use with flat sheet filters, use the following procedure:

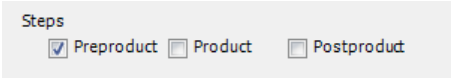
Step	Action
1	In the ***Basic Settings*** window, select ***Filter Type →Flat Sheet***.
2	In the ***Method*** drop-down list, select ***Concentration\Diafiltration***.



3	In the ***Filter List***, ***User-defined filter*** is auto selected.
4	In the next list, select ***Concentration/Diafiltration***.



Step	Action
5	Select ***Preproduct*** process step.



6 The ***Flat Sheet (specification per filter)*** displays the default values for a user-defined flat sheet cassette. The end user must enter values for the following information:

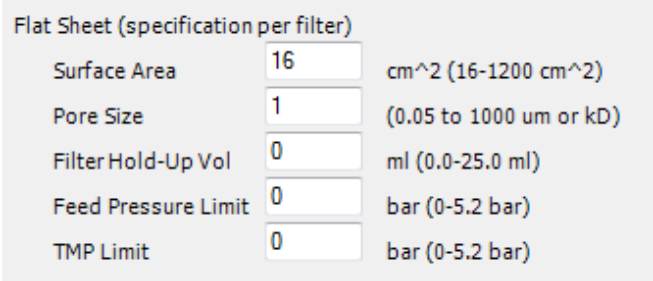
a. ***Surface Area***

b. ***Pore Size***

c. ***Filter Hold-Up Vol***

d. ***Feed Pressure Limit***

e. ***TMP Limit***

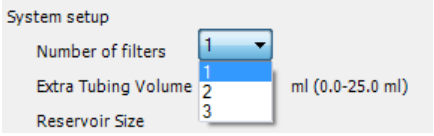


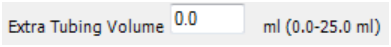
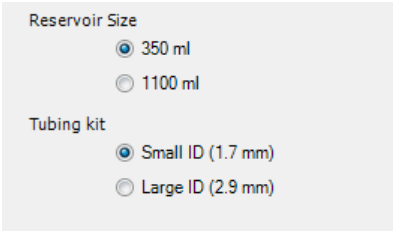
This information is usually available from the manufacturer.

Note:

When using TMP as the filtration control mode in a method, always choose a TMP value in the product steps that is below the TMP limit. Otherwise, the TMP limit may lead to a TMP pressure alarm, which will pause the run.

7 If several filters are assembled together in parallel, select in the ***System Setup*** section the number of filters (1-3). If only one filter is used, keep the default value of 1.

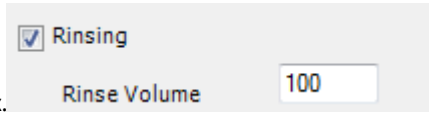


Step	Action
8	If any extra tubing is used on the recirculation loop, calculate the extra volume added and enter the value in the ***Extra Tubing Volume*** box. 
9	Select the reservoir size and tubing kit i.d. used in the recirculation loop. 

6.3.2 Preproduct step dialog

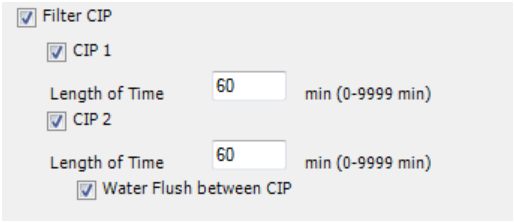
Rinsing

To include a rinsing step in the method, use the following procedure:

Step	Action
1	Check the Rinsing box. 
2	Select the rinsing volume; this is the volume of water which will be flushed through the filter into the permeate. The default value is 2 mL per cm^2 of filter surface area.

Filter CIP

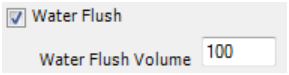
To include a filter CIP step in the method, use the following procedure:

Step	Action
1	Check the ***Filter CIP*** box.
2	Select either a one- or two-step CIP, with an optional water flush in between.
3	Enter the desired CIP circulation time in the ***Length of Time*** box. 

Water flush

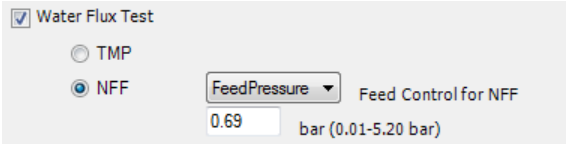
To include a water flush step in the method, use the following procedure:

Step	Action
1	Check the ***Water Flush*** box.

Step	Action
2	Select the flush volume; this is the volume of water which will be flushed through the filter into the permeate. The default value is 2 mL per cm^2 of filter surface area.  ***Note:*** *After a filter CIP, it is recommended to run a water flush.* ***Note:*** *If a water flux test will be performed,* ***Rinsing*** *or* ***Water Flush*** *should be performed just before the water flux test.*

Water flux test

To include a water flux test in the method, use the following procedure:

Step	Action
1	Check the ***Water Flux Test*** box.
2	Select ***TMP*** or ***NFF*** (Normal Flow Filtration) as control mode. **a.** The default TMP setting for all ultrafilters is 1 bar. For high molecular weight hollow fiber ultrafilters (≥ 500 kD), it is recommended to set this value to 0.5 bar.  **b.** NFF (feed pressure) is default for microfiltration hollow fibers with a cut off of 0.1 µm and larger.  **c.** NFF control of feed flow is also possible. ***Note:*** *When comparing status of a filter as a function of time and number of experiments, we recommend using the same filtration mode parameter each time you run the water flux test.*

Buffer conditioning

To include a buffer conditioning step in the method, use the following procedure:

Step	Action
1	Check the ***Buffer Conditioning*** box.
2	Select the buffer rinse volume; this is the volume of buffer which will be flushed through the filter into the permeate. The default value is 30 mL. This can be edited to a maximum value of 300 mL for the small reservoir or 1000 mL for the large reservoir.  ***Note:*** *If a CIP has been performed, we recommend performing a buffer conditioning after the water flush to ensure that the pH in the system is suitable for any following product steps.*

6.3.3 Visualization of the preproduct steps

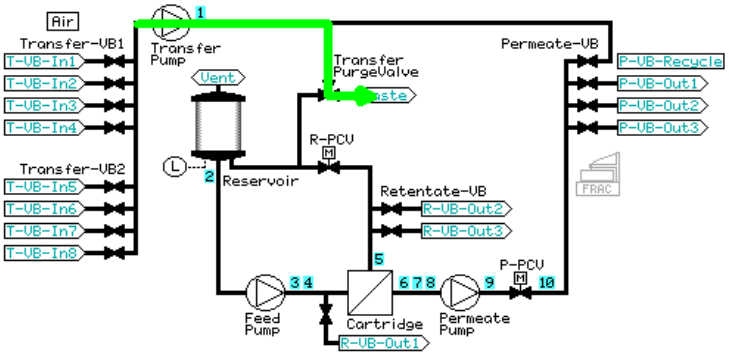
For information on specific instructions, for example ***Constant Retentate Volume***, see *Chapter 14 Strategy instructions, on page 268*.

Depending on the previous step, some of the steps begin with a ***Prepare System*** or ***Prepare System and Reservoir*** block.

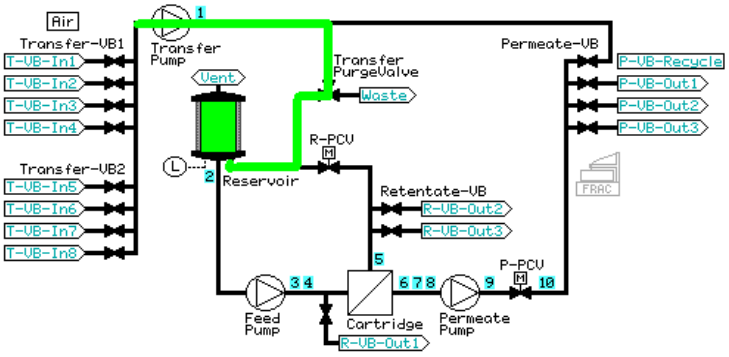
Prepare System and Reservoir

The ***Prepare System and Reservoir*** step fills and empties the reservoir completely and thoroughly flushes the recirculation loop. The inlet valve position chosen depends on the liquid of the specific step.

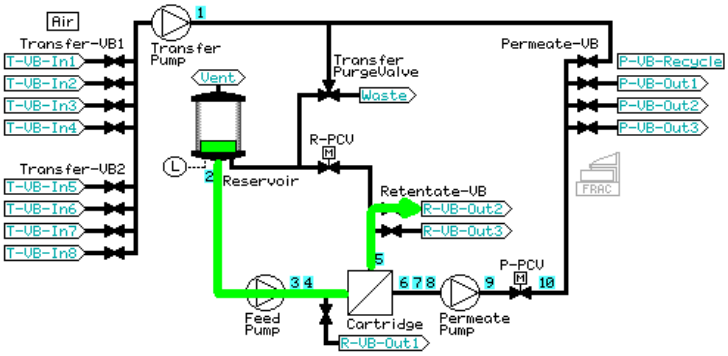
Stage	Description
1	The transfer inlet used is primed to waste through the transfer purge valve.



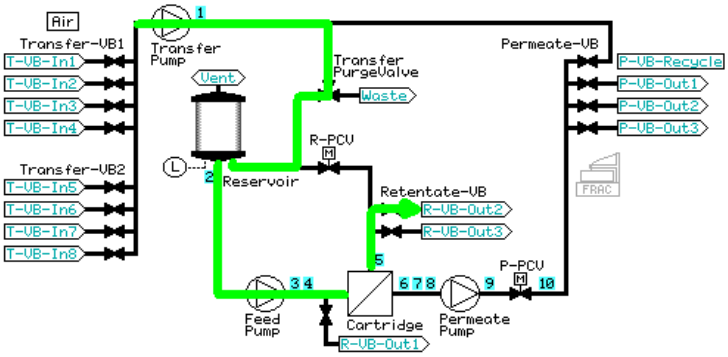
2	The reservoir is filled to the maximum volume (350 mL for the small reservoir, 1100 mL for the large reservoir).



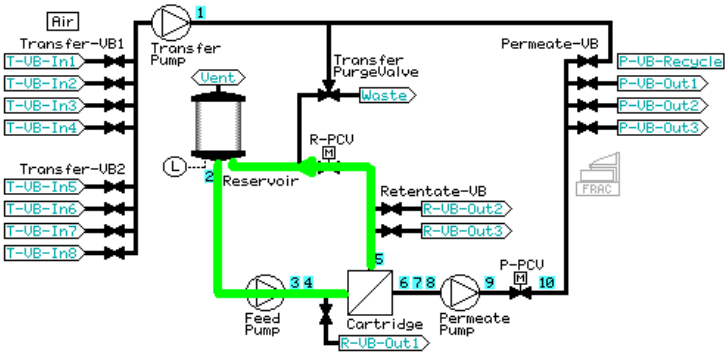
Stage	Description
3	The reservoir is emptied through **R-VB-Out2** (waste).



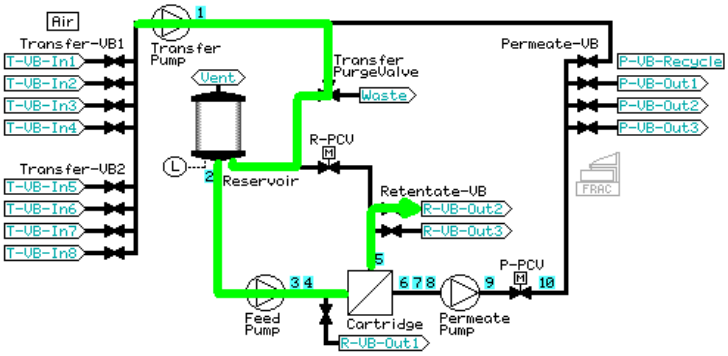
4	The reservoir is filled to a minimum working volume. A low transfer and equal feed flow is set, and 50 mL is pumped out of the retentate to waste through **R-VB-Out2**.



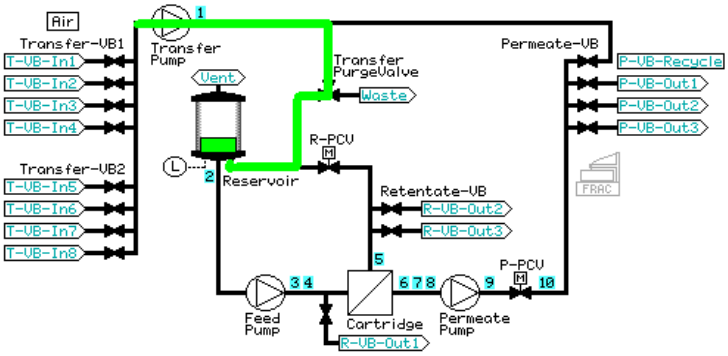
5	The tubing in the pathway between **R-VB-Out2** and the reservoir is rinsed by recirculating at a low feed flow rate for less than a minute.

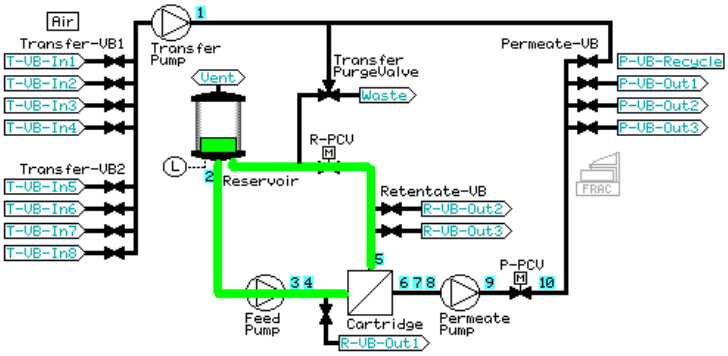
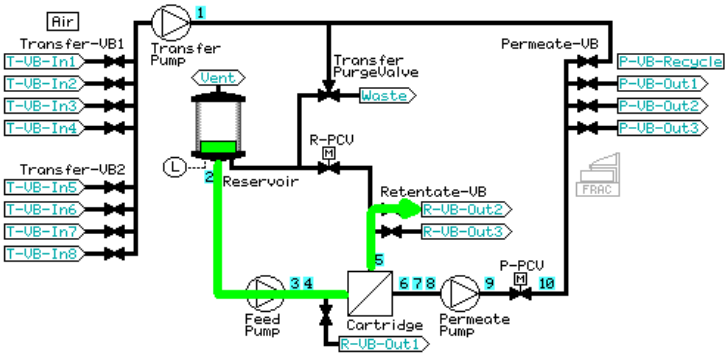


Stage	Description
6	The reservoir is emptied and refilled with the minimum working volume.
7	A low transfer and equal feed flow is set, and 50 mL is pumped out of the retentate to waste through **R-VB-Out2**.



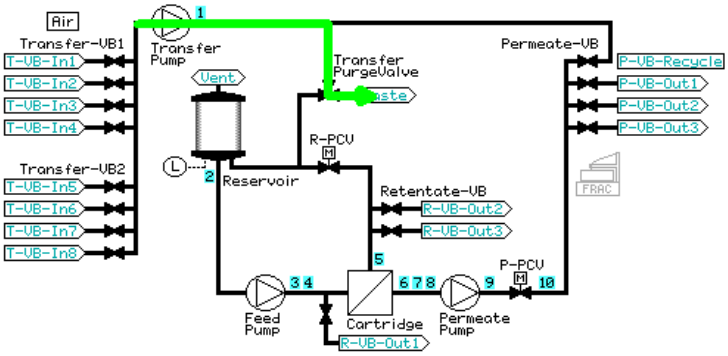
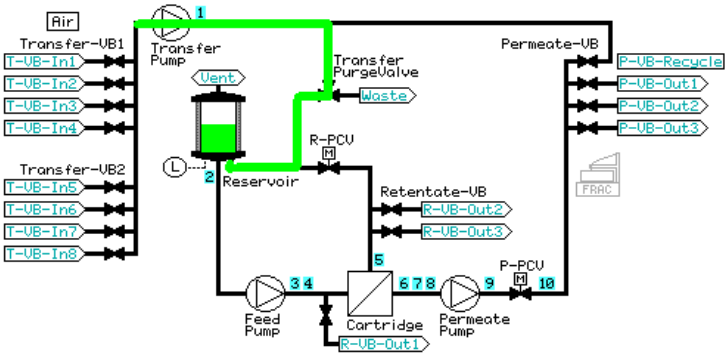
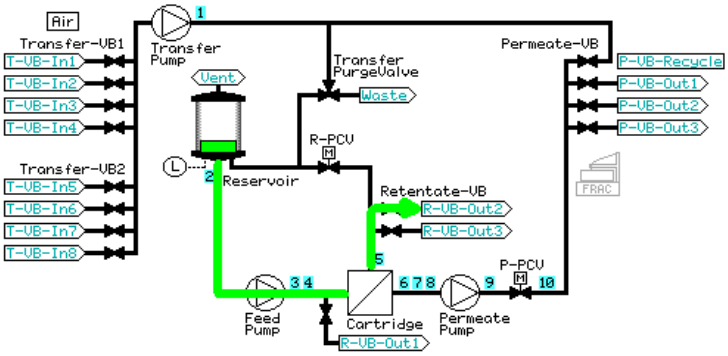
8	The reservoir is filled to a low volume.

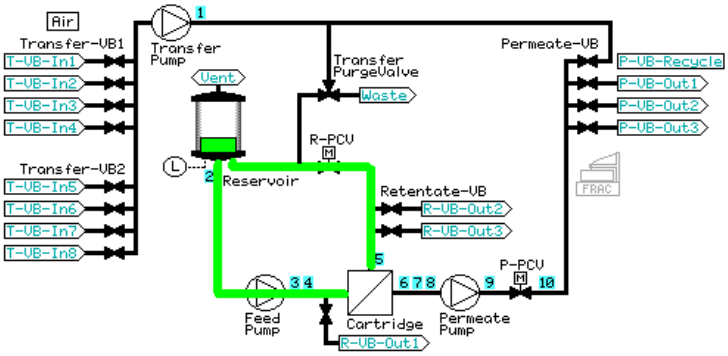
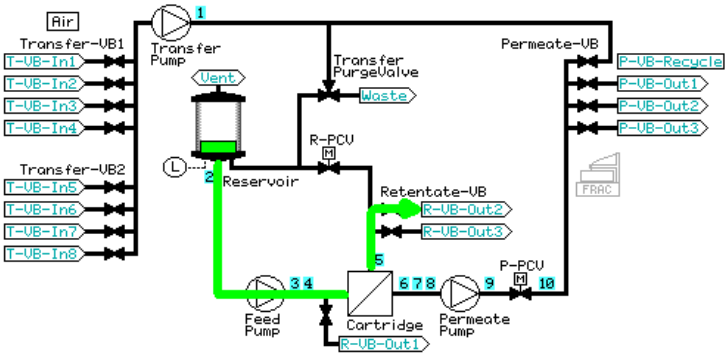


Stage	Description
9	The retentate is recirculated for one minute using a feed pressure control of 80% of the maximum feed pressure value (to a maximum of 3 bar). 
10	The reservoir is emptied through **R-VB-Out2**. 

Prepare system

The ***Prepare System*** step performs a flush of the recirculation loop.

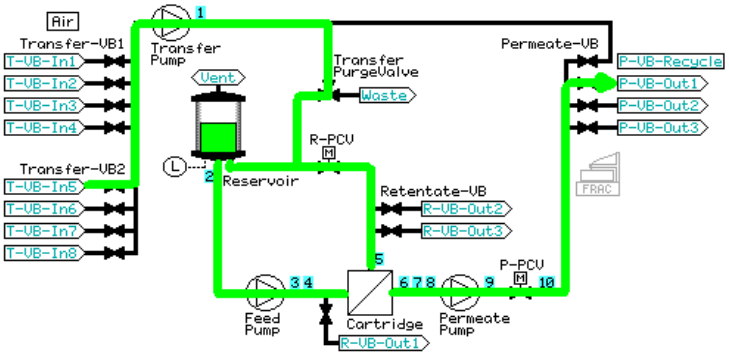
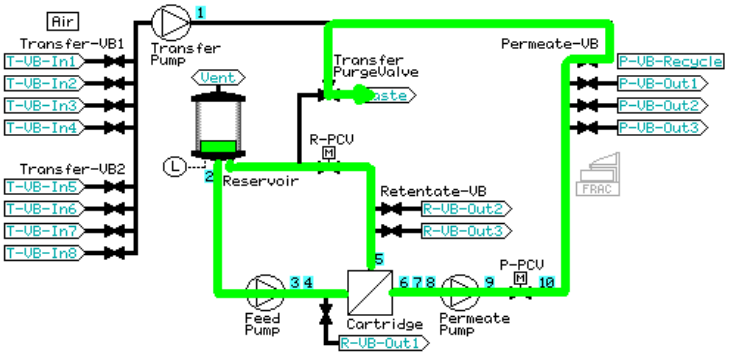
Stage	Description
1	The transfer inlet used is primed to waste through the transfer purge valve. 
2	The reservoir is filled to a low volume. 
3	The retentate is rinsed out **R-VB-Out2** for the hold-up volume plus 5 mL. 

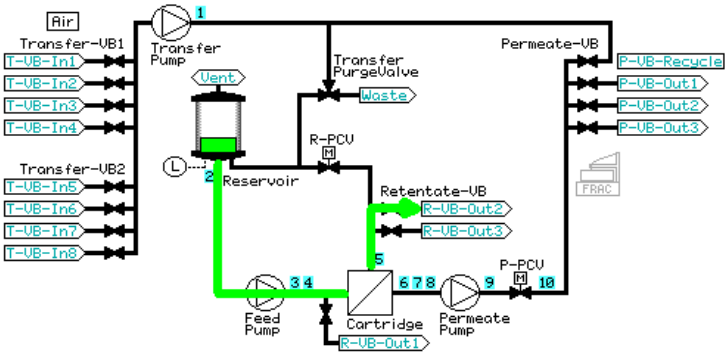
Stage	Description
4	The retentate is recirculated for one minute using a feed pressure control of 80% of the maximum feed pressure value (to a maximum of 3 bar). 
5	The reservoir is emptied through **R-VB-Out2** (Waste). 

Rinsing

The ***Rinse*** step rinses storage solution from the filter.

Stage	Description
1	The system is prepared according to the procedure described in *Prepare system, on page 112*. The transfer inlet used is **T-VB-In5** (water).

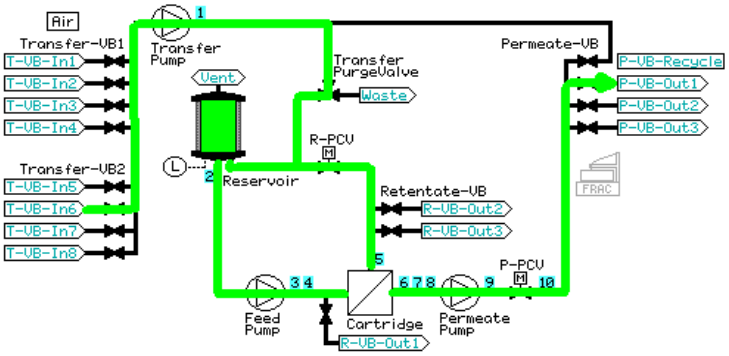
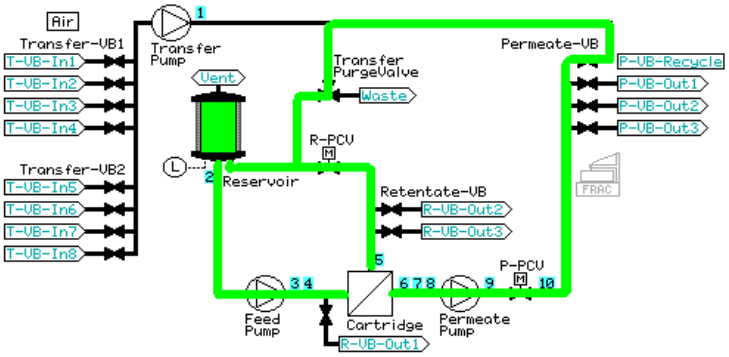
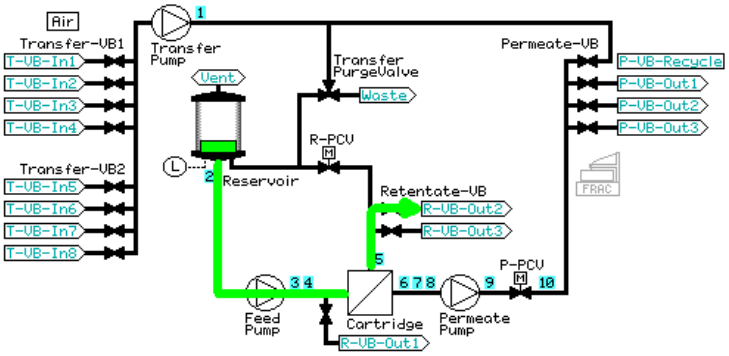
Stage	Description
2	The reservoir is filled with 100 mL water. ***Constant Retentate Volume*** is activated. The crossflow rate is set at a feed pressure 80% of the maximum feed pressure value (to a maximum of 3 bar) and TMP regulation of 1 bar is started. The volume input in the dialog is rinsed through the filter out **P-VB-Out1** (Waste). 
3	***Constant Retentate Volume*** is disabled. 10 mL of the reservoir volume is emptied through the permeate recycle (**P-VB-Recycle**) to waste (**Transfer_Purge_Valve Waste**). 

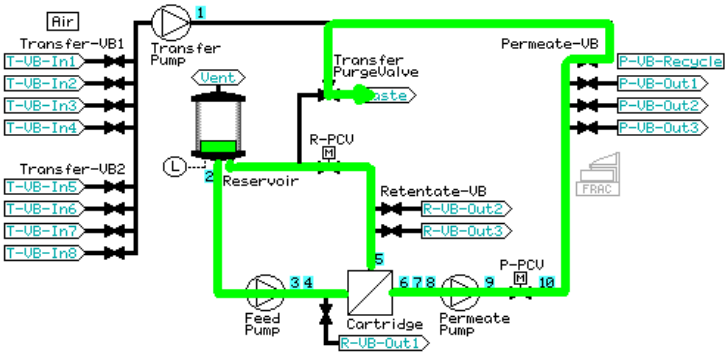
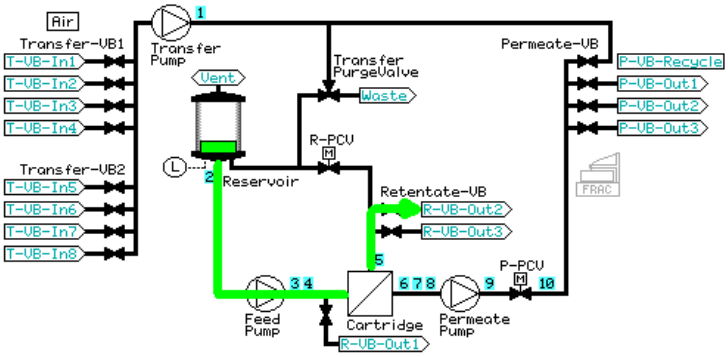
Stage	Description
4	The rest of the reservoir volume is emptied through **R-VB-Out2** and the rinsing is complete. 

Filter CIP

The ***Filter CIP*** step circulates cleaning solution to clean the system and the filter.

Stage	Description
1	The system is prepared according to the procedure described in *Prepare system, on page 112*. The transfer inlet used is **T-VB-In6** or **T-VB-In7** (CIP solution), depending on whether 1 or 2 CIP steps were selected.
2	The reservoir is filled with CIP solution to either the maximum volume (small reservoir, 350 mL) or a specified fill volume (large reservoir, minimum 200 mL (default) to 1100 mL).
3	***Constant Retentate Volume*** is activated and the permeate valve is opened to **P-VB-Out1**. The crossflow rate is set at a feed pressure of 80% of the maximum feed pressure value (to a maximum of 3 bar) and flux rate filtration control of 30 LMH (flat sheet cassette, HF microfilter) or TMP filtration control of 1 bar (HF ultrafilter) is started.

Stage	Description
4	The first 30 mL passes through the membrane out **P-VB-Out1**. 
5	After 30 mL has passed through the membrane, the permeate valve is set to **P-VB-Recycle** and the liquid is recycled back into the reservoir for the specified recirculation time. 
6	After the specified CIP recirculation time, ***Constant Retentate Volume*** is disabled and the reservoir is emptied through **R-VB-Out2**. 

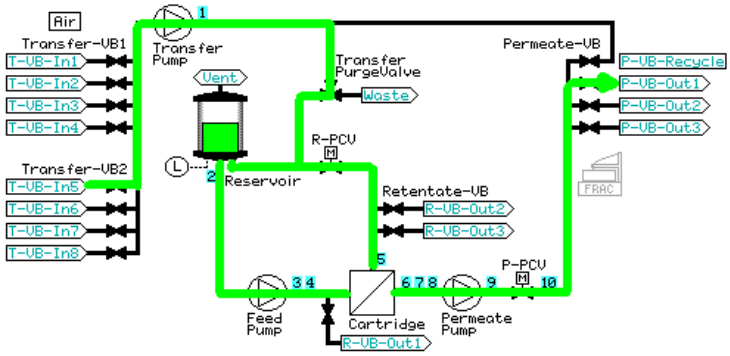
Stage	Description
7	If a ***Water Flush between CIP*** has been selected, the system is prepared according to the procedure described in *Prepare system, on page 112*. The transfer inlet used is **T-VB-In5** (water).
8	The reservoir is filled with 100 mL of water and 10 mL of the reservoir volume is emptied through the permeate recycle (**P-VB-Recycle**) to waste (**Transfer_Purge_Valve Waste**). 
9	The reservoir is then emptied through **R-VB-Out2**. 
10	If a CIP 2 step has been selected, the system is prepared according to the procedure Prepare System described in *Prepare system, on page 112*. The transfer inlet used is **T-VB-In7** (CIP 2 solution).
11	The reservoir is filled with either 100 mL CIP 2 solution (small reservoir) or the specified fill volume (large reservoir) and the procedure described above in steps 3 to 6 is repeated.

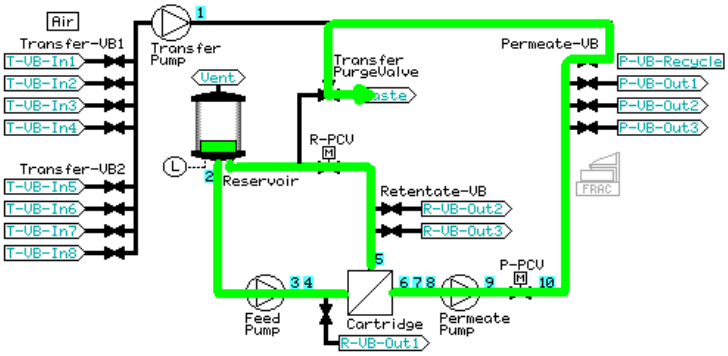
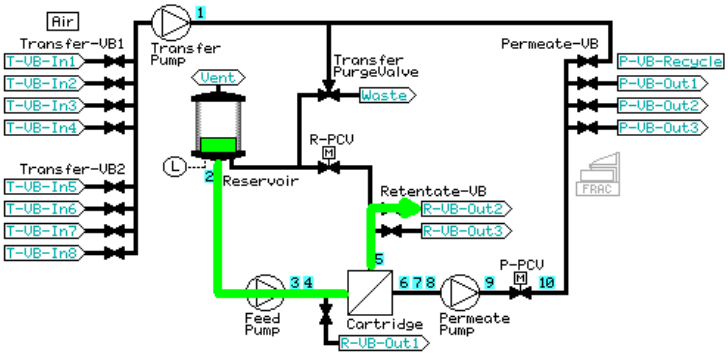
Water flush

If the previous step was a filter CIP, the system and reservoir are thoroughly flushed according to the procedure described in *Prepare System and Reservoir, on page 109*

Otherwise, the system is prepared according to the procedure described in *Prepare system, on page 112*. The transfer inlet used is **T-VB-In5** (water).

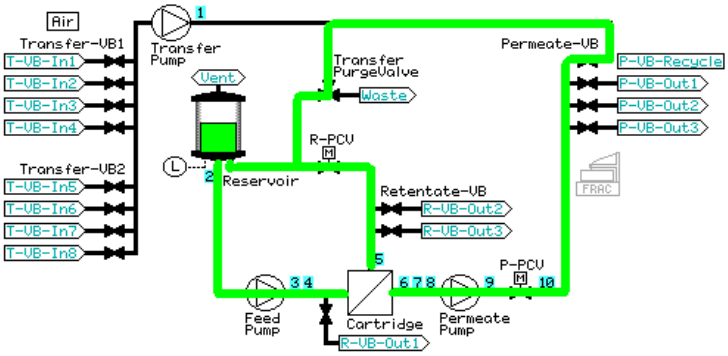
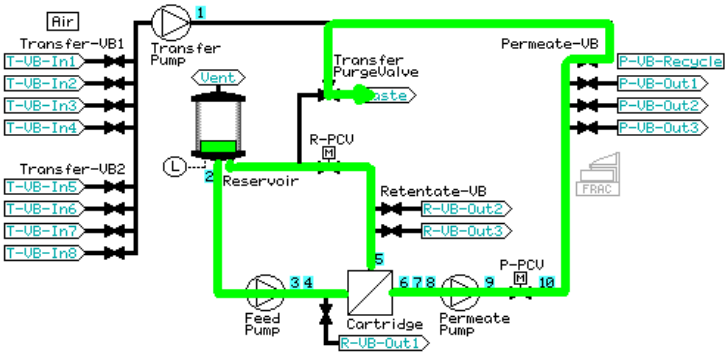
Stage	Description
1	The reservoir is filled with 100 mL water. ***Constant Retentate Volume*** is activated, and the permeate valve is opened to **P-VB-Out1**. The crossflow rate is set at a feed pressure 80% of the maximum feed pressure value (to a maximum of 3 bar) and flux rate filtration control of 30 LMH (HF microfilter) or TMP filtration control of 1 bar (HF ultrafilter, flat sheet cassette) is started. The filter is flushed with specified flush volume out **P-VB-Out2** (default volume is 2 mL per cm^2 of filter surface area).

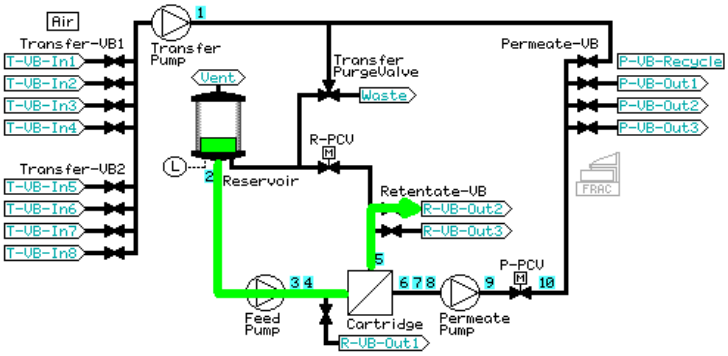


Stage	Description
2	After the specified flush through the filter, ***Constant Retentate Volume*** is disabled. If a ***Water Flux Test*** has been selected, the system skips to the test; if not, 10 mL is emptied through the filter. **P-VB** is set to ***Recycle*** and the liquid leaves the system through **Transfer_Purge_Valve Waste**. 
3	The reservoir is emptied through **R-VB-Out2**. 

Water flux test

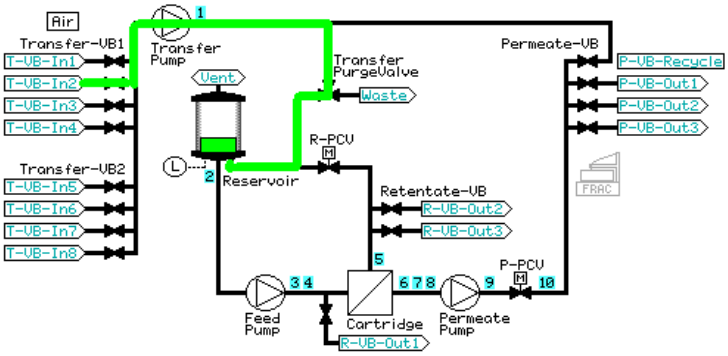
If the step prior to the water flux test is a water flush step, the reservoir remains filled and the ***Water Flux Test*** is a continuation of the ***Water Flush*** step. If the water flux test is used as a stand-alone step, the system is prepared according to the procedure described in *Prepare system, on page 112*. The transfer inlet used is **T-VB-In5** (water). The reservoir is filled with 50 mL water, ***Constant Retentate Volume*** is activated, and the permeate valve is opened to **P-VB-Out1**.

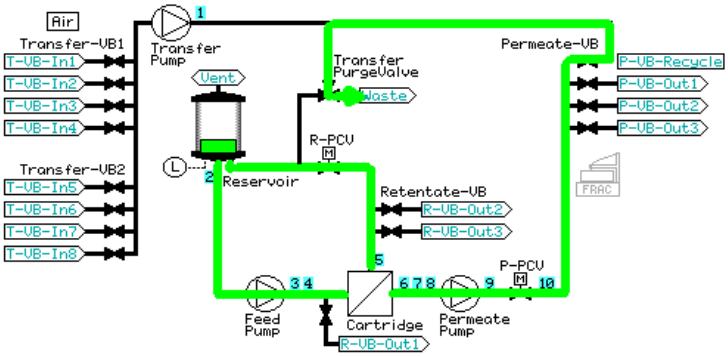
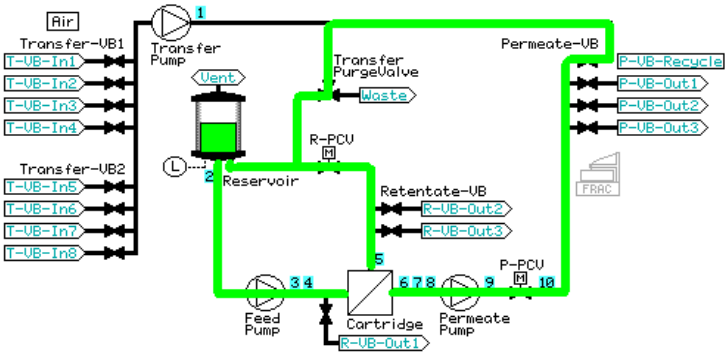
Stage	Description
1	**P-VB** is set to **Recycle** and the **Transfer_Purge_Valve** is set to **Reservoir**. A permeate flow is started by the specified TMP control (HF ultrafilter, flat sheet cassette; default value 1 bar) or in NFF mode (HF microfilter; default value is feed pressure, determined by the filter pore size). When a stable flux has been achieved, the normalized water flux value is measured by setting a ***Set_Eval_Mark*** with the parameter ***Normalized_Water_Flux***. 
2	Before ending the step, 10 mL of water is flushed through the filter. **P-VB** is set to **Recycle** and the liquid leaves the system through **Transfer_Purge_Valve Waste**. 

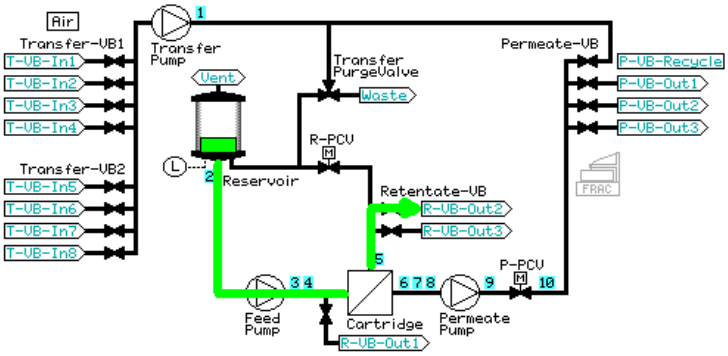
Stage	Description
3	The reservoir is then emptied through **R-VB-Out2**. 

Buffer conditioning

The ***Buffer conditioning*** step conditions filter and system components before adding product to minimize adverse chemical reactions.

Stage	Description
1	The system is prepared according to the procedure described in *Prepare system, on page 112*. The transfer inlet used is **T-VB-In2** (conditioning buffer). The reservoir is filled with a small volume of buffer. 

Stage	Description
2	**P-VB** is set to **Recycle** and the **Transfer_Purge_Valve** is set to **Waste**. The crossflow rate is set at a feed pressure 80% of the maximum feed pressure value (to a maximum of 3 bar) and flux rate filtration control of 30 LMH (HF microfilter) or TMP filtration control of 1 bar (HF ultrafilter, flat sheet cassette) is started. The specified buffer rinse volume is flushed through the filter into the permeate (default value is 30 mL, but this can be edited to a maximum value of 300 mL for the small reservoir or 1000 mL for the large reservoir). 
3	The **Transfer_Purge_Valve** is switched to **Reservoir** and the buffer is recycled for 5 minutes. 

Stage	Description
4	The reservoir is emptied through **R-VB-Out2**. 

7 Create product steps using the Method Wizard

About this chapter

This chapter provides information on how to create product steps for ultrafiltration and microfiltration using the ***Method Wizard***.

In this chapter

7.1 Ultrafiltration

About this section

This section provides information on how to create product methods for Ultrafiltration using the ***Method Wizard***.

In this section

7.1.1 Introduction

Concentration

During a concentration step, the volume of sample in the reservoir is reduced. The product is retained at the retentate side of the membrane.

If the sample volume is larger than the reservoir volume, the reservoir can be continuously fed with sample solution (***Fed Batch***).

Diafiltration

Diafiltration is a filtration process used for the removal of smaller ionic solutes, in which one buffer is removed and replaced with an alternative buffer (***Buffer Exchange***). The product is retained at the retentate side.

A buffer exchange is typically run after a concentrating step using the same filter. This allows for the combination of concentration and diafiltration into one method, using one filter.

If no concentration is performed before a diafiltration step, for example, when diafiltration is the only step, the hold-up volume on the retentate side will dilute the sample. Therefore, if performing a diafiltration first, it is recommended to perform an initial concentration, especially when the sample volume is low. Enter a ***Concentration Factor*** of 1 as the end point. See *Concentration endpoint, on page 134*.

Product recovery after Concentration/Diafiltration

There are two alternatives to recover the product in the retentate:

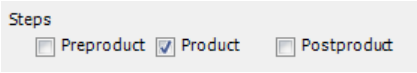
- **No Recovery**: select this option if the retentate volume will be recovered manually. The system will enter a Hold with a low recirculation rate to prevent sedimentation, allowing the user to either recover immediately, or end the method and then recover.
- **Recovery**: the reservoir is first emptied through **R-VB-Out3**, followed by a buffer chase, allowing the maximum volume of undiluted sample to be recovered. A defined number of flushes can then be selected and the retaining dilute product will be emptied through **R-VB-Out1**.

For descriptions of the Recovery procedures, see *Section 7.1.6 Recovery dialog, on page 141*.

7.1.2 Basic settings dialog

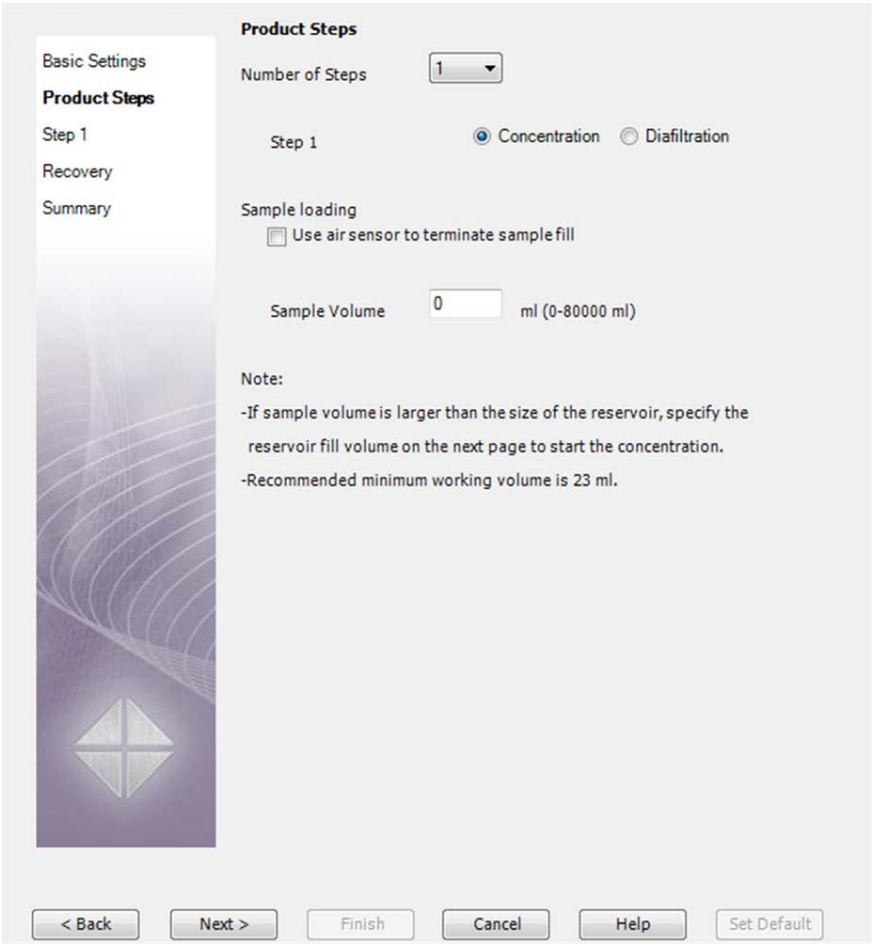
Input the basic settings (filter type, specifications, tubing kit i.d., and size of reservoir) as detailed in *Section 6.3.1 Basic settings dialog, on page 100*.

Select ***Product*** in the ***Steps*** selection.



7.1.3 Product steps dialog

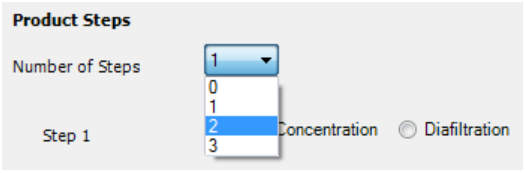
The following illustration shows the product steps dialog screen.



In the ***Product Steps*** dialog, a minimum working volume is displayed. The minimum working volume is the system hold-up volume including the filter hold-up, with an addition of a small volume in the reservoir. This volume depends on the reservoir size, tubing kit, and filter volume. This is the lowest working volume that is recommended.

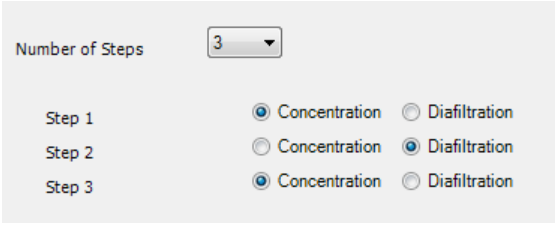
Note: *Minimum working volume will vary with reservoir size and tubing kit used and is not the same as the system hold-up volume. To maintain smooth processing, a small volume is added to the system hold-up volume in the calculation of minimum working volume. For information on system hold-up volume, see Appendix A.*

To set the concentration and diafiltration parameters, follow the steps below.

Step	Action
1	In the ***Product Steps*** dialog, select up to 3 concentration and diafiltration steps. 

Note:

For diafiltration, only 2 steps can be selected with any combination of steps; this is due to the limited number of transfer valve block inlets that are used as standard for diafiltration buffer.

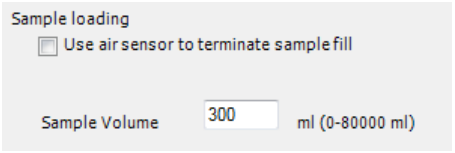


Step	Action
2	Enter the sample volume. There are 3 options:

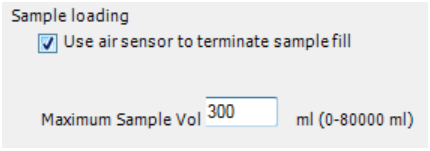
a. Enter a set volume.

Note:

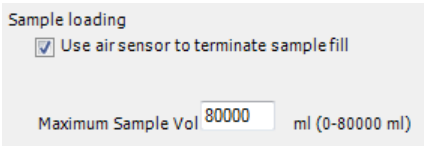
If you enter a volume greater than the maximum volume of the chosen reservoir, on the next page a ***Fill Volume*** *must be entered, and the system will operate in* ***Fed Batch*** *mode. In this case, the reservoir is continually refilled as a function of the permeate flow. This is possible only when concentration is the first step. If the total sample volume fits into the chosen reservoir size, the system will operate in* ***Tank Batch*** *mode.*



b. Enter a set volume and check ***Use air sensor to terminate sample fill***. This will activate the air sensor found on **T-VB-In1**. If air is detected before the sample volume is reached, the air sensor terminates the sample load, but if no air is detected before the sample volume is reached, the sample volume terminates the load.

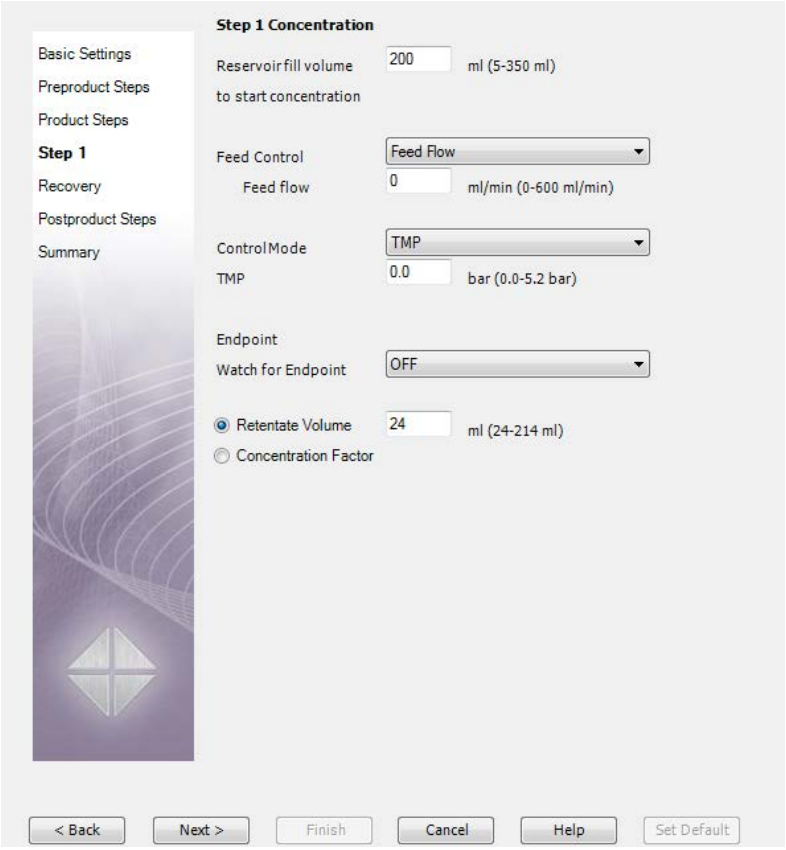


c. Check ***Use air sensor to terminate sample fill*** but leave the sample volume on the default of 80 000 mL. In this case, only air detection on **T-VB-In1** will terminate the sample load.

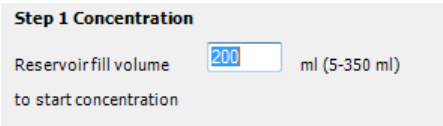


7.1.4 Concentration step dialog

The following illustration shows the concentration steps dialog screen.



If a sample volume that is greater than the chosen reservoir volume (e.g., fed batch operation) or if the ***Use air sensor to terminate sample fill*** option has been selected, the reservoir fill volume must be entered. The default fill volume is 200 mL for either reservoir but the maximum fill volume will depend on the size of the reservoir chosen. A reservoir minimum is also displayed; this is the minimum recommended working volume for the reservoir (5 mL for the small reservoir, 8 mL for the large reservoir).

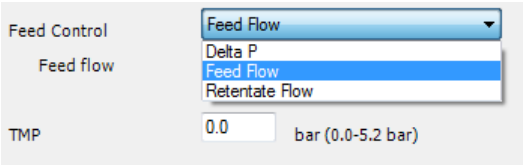


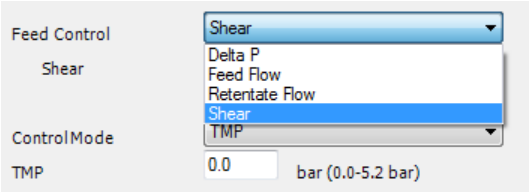
Next step: Set the crossflow rate and filtration control mode.

Feed and filtration control

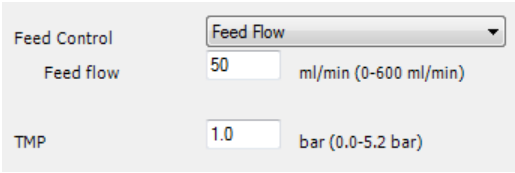
There are four ways to create the crossflow, all of which are controlled by the feed pump:

- Constant ***DeltaP***
- Constant ***Feed Flow***
- Constant ***Retentate Flow***
- ***Shear*** rate (only when hollow fibre has been selected)





Step	Action
1	Enter the value for the selected crossflow rate, for example a ***Feed Flow*** of 50 mL/min. ***Note:*** *When using shear rate as crossflow rate for hollow fibers, the feed flow rate that equals a chosen shear rate must be calculated in advance. The maximum feed flow rate on the system is 600 mL/min, and if a shear rate for the selected hollow fiber exceeds this, the feed pump will run at 600 mL/min. No error or alarm will be generated. For more information on shear rates, and conversion to feed flow rate for a hollow fiber, refer to the Hollow fiber operating guide.*

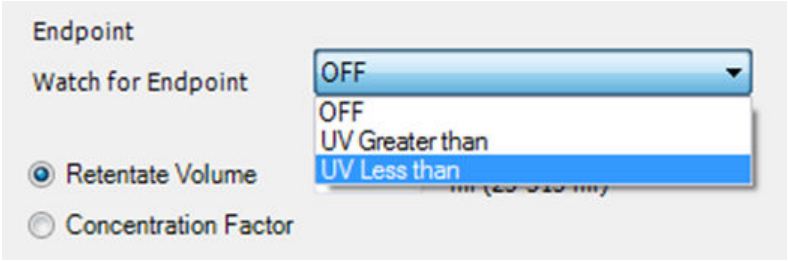
Step	Action
2	Enter the desired TMP control value in bar.  ***Note:*** *For ultrafilter hollow fibers and flat sheet cassettes, the only filtration control mode available is TMP control. This can be edited to permeate flux control in the* ***Text Editor*** *after the method has been created.* ***Note:*** *When using TMP as the filtration control mode in a method, always choose a TMP value in the product steps that is below the TMP limit. Otherwise, the TMP limit can lead to a TMP pressure alarm, which will pause the run.*

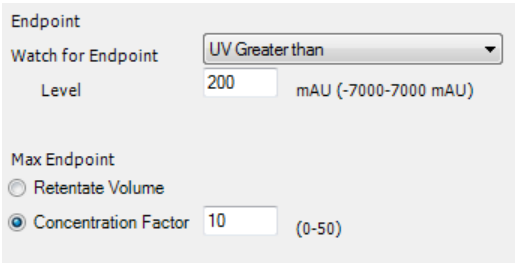
Concentration endpoint

There are two ways to end a concentration step:

- ***Watch for Endpoint***
- ***Retentate Volume/Concentration Factor***

To place a watch on the UV to end the concentration step, use the following procedure:

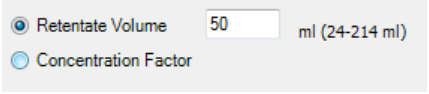
Step	Action
1	Select ***UV Greater than*** or ***UV Less than*** from the drop-down list. 

Step	Action
2	Enter an endpoint value in the ***Level*** window. UNICORN will end the concentration step based on the specified UV signal (in mAU) in the permeate. ***Note:*** *If the **Watch for Endpoint** setting is used, a maximum endpoint must still be designated. UNICORN will end the concentration step when either the UV level or maximum endpoint is met.*
3	Set a maximum endpoint value for either ***Retentate Volume*** or ***Concentration Factor***. 

Step	Action
1	Set the ***Watch for Endpoint*** option to ***OFF***.
2	Under ***Max endpoint***, select either ***Retentate Volume*** or ***Concentration Factor***.

Step	Action
3	Enter the value for the endpoint:

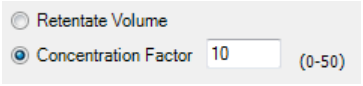
a. If a ***Retentate Volume*** is the desired endpoint, an applicable range will be given which depends on the total retentate hold-up volume, the fill volume, and the reservoir size.



Note:

It is not possible to end a concentration step on a volume greater than the fill volume plus the hold-up volume minus 5 mL. There is also a minimum retentate volume equal to the retentate hold-up volume plus 5 mL. This makes sure that a small amount of liquid remains in the reservoir at the end of the concentration step.

b. If a ***Concentration Factor*** is the desired endpoint, a concentration factor between 1 and 50 must be entered.



Note:

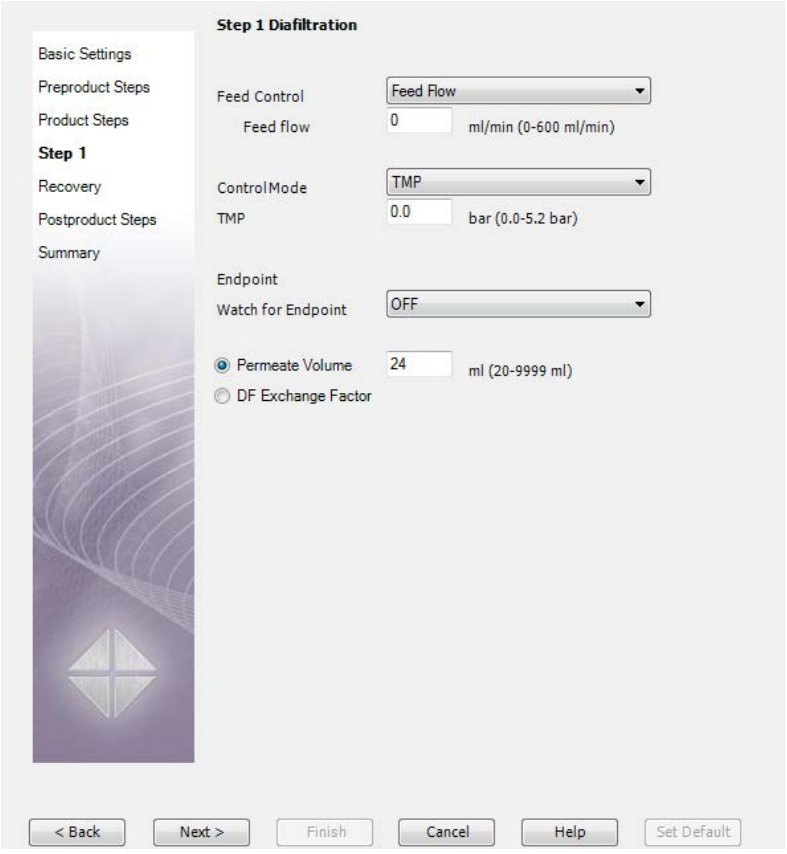
The expected sample volume and concentration factor must be estimated to make sure that they are achievable with the reservoir volume and retentate hold-up volume. For example, if you have an expected sample volume of 1000 mL, and are using the small reservoir, it is not possible to achieve a concentration factor of 2. The system will however not give an error message or alarm, but will end the concentration when the sample load reaches twice the fill volume plus the hold-up volume. Also, you may not be able to reach a desired concentration factor due to system minimum working volume limitation. For example, if the retentate hold-up volume is 25 mL, the sample volume is 100 mL, and the desired concentration factor is 5, the system's catastrophic ReservoirEmpty alarm will pause the system when the level sensor detects an empty reservoir at 25 mL. It is recommended to use a minimum reservoir volume of 5 mL, although more may be required depending on crossflow rate.

Note:

A concentration factor of 1 means no concentration, which can be used to compensate for an initial dilution due to liquid in the recirculation loop on the retentate side. This can be utilized in the diafiltration of small volumes as a planned single step. *See Diafiltration, on page 127.*

7.1.5 Diafiltration step dialog

If diafiltration of a small volume is planned, it is recommended to perform a concentration as an initial step. This is to avoid dilution of the sample due to the hold-up volume on the retentate side. Note also the sample volume limitation when performing a diafiltration as a first step. For more information, see *Diafiltration, on page 127* and *Concentration endpoint, on page 134*.



Feed and filtration control

See *Feed and filtration control, on page 133*.

Diafiltration endpoint

There are two ways to end a diafiltration step:

- ***Watch for Endpoint***
- ***Permeate Volume/Diafiltration Exchange Factor***

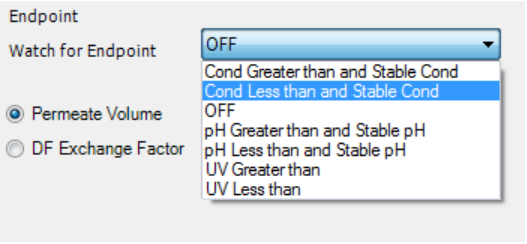
Three signals can be monitored to end a diafiltration step:

- conductivity
- pH
- UV

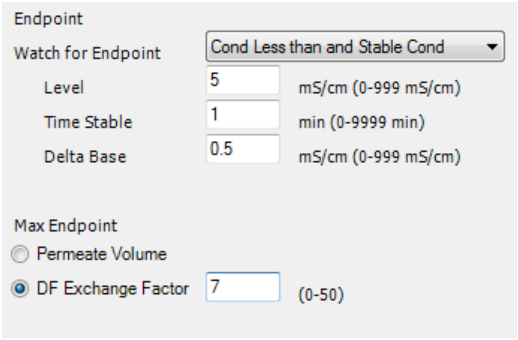
Note: *A maximum volume endpoint must be set using the **Max Endpoint** setting even if a **Watch for Endpoint** condition has been set. UNICORN will end the concentration step when either the monitor condition or maximum endpoint condition is met.*

Watch for endpoint

To place a watch to end the diafiltration step, use the following procedure:

Step	Action
1	In the ***Watch for Endpoint*** drop-down menu, select the signal and condition from the following options: **a.** ***Conductivity Greater than and Stable Conductivity*** **b.** ***Conductivity Less than and Stable Conductivity*** **c.** ***pH Geater than and Stable pH*** **d.** ***pH Less than and Stable pH*** **e.** ***UV Greater than*** **f.** ***UV Less than*** 
2	*Either:* If UV endpoint has been selected, enter the monitor signal level at which the diafiltration step should stop, *or* If conductivity or pH endpoint has been selected, enter the monitor signal level at which the monitoring of the stable signal should start.

Step	Action
3	Enter the time the signal should be stable for the watch to be met (conductivity or pH).
4	Enter ***Delta Base***, the allowed fluctuation (+ and – value) for the signal to be considered stable (conductivity or pH).

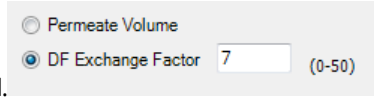


Note: *To end the diafiltration with a watch on pH, or UV signal, a maximum volume endpoint must still be designated. UNICORN will end the concentration step when either the monitor condition or maximum endpoint condition is met.*

Permeate Volume/Diafiltration Exchange Factor

To end the diafiltration step based on either a permeate volume or a diafiltration exchange factor:

Step	Action
1	Set the ***Watch for Endpoint*** option to ***OFF***.
2	Select either ***Permeate Volume*** or ***DF Exchange Factor***.

Step	Action
3	Enter the value for the endpoint: **a.** If a ***Permeate Volume*** is the desired endpoint, enter the total permeate volume to end the diafiltration.  **b.** If a ***Diafiltration Factor*** is the desired endpoint, a value between 0 and 50 must be entered. 

Note: *Due to possible unknown sample volume when loading based on air detection, UNICORN cannot estimate the required volume of diafiltration buffer. Make an approximate calculation to make sure that there is enough diafiltration buffer to achieve the desired diafiltration exchange factor. If the system runs out of diafiltration buffer, the sample will concentrate until the catastrophic* ***Reservoir Empty*** *alarm pauses the system.*

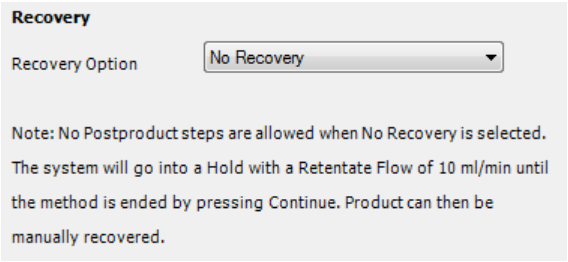
7.1.6 Recovery dialog

Recovery options

There are two recovery options: ***Recovery*** or ***No Recovery***.

If ***No Recovery*** is selected, the system will go into a ***Hold*** with a retentate flow rate of 10 mL/min to avoid sedimentation. The material can be recovered manually either during this ***Hold*** step, or select ***Continue*** in ***System Control*** to end the method. The sample can then be recovered as desired.

Note: *If you opt for* ***No Recovery*** *then no postproduct steps are allowed.*



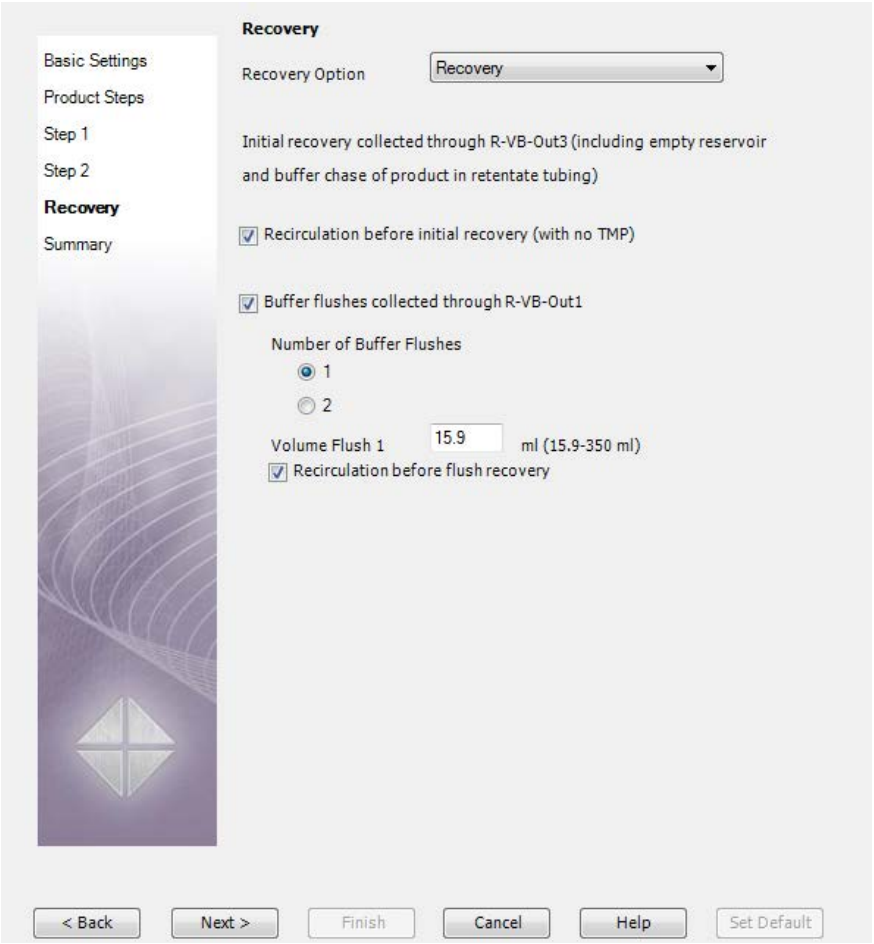
If ***Recovery*** is selected, the reservoir can be emptied by the following ways.

For flat sheet casettes, the reservoir is emptied through **R-VB-Out3**.

For hollow fibers, under ***Retentate Oulet*** select either:

- ***ProductFlush***: the initial recovery is collected through **R-VB-Out3** *or*
- ***Waste***: the retentate is sent to waste through **R-VB-Out2**

If ***Waste*** is chosen, the product steps are terminated after an optional 5-minute re-circulation with open TMP valve.



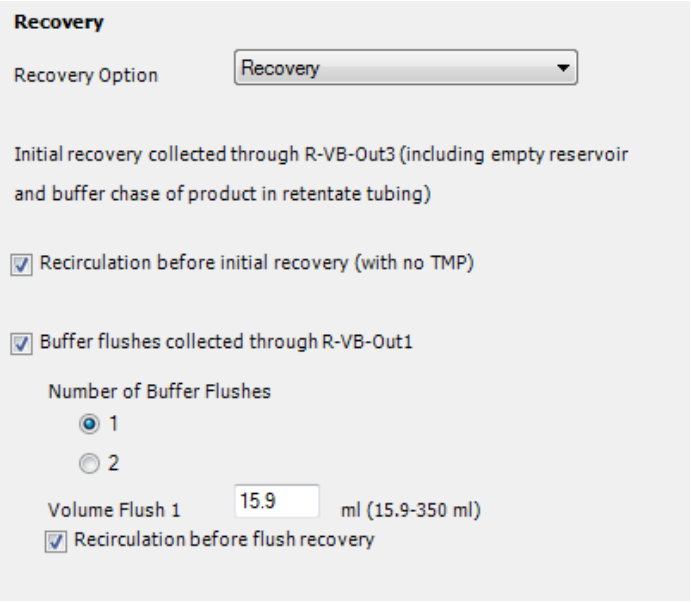
Note: *If an old type retentate valve is used on the system, make sure that any recovery vessels are not placed lower than the reservoir, as siphoning can occur.*

If ***Recirculation before initial recovery (with no TMP)*** is checked, the retentate is recirculated with an open **R-PCV** (TMP) valve for 5 minutes, to aid in the recovery process. This recirculation sweeps any proteins bound to the membrane (concentration polarisation) or gel layer that formed during the concentration and diafiltration process back into the bulk flow to enhance the yield. The retentate is then recovered through **R-VB-Out3**, followed by a buffer chase This allows recovery of the undiluted product in the reservoir and 70% of the undiluted material in the recirculation pathway to the **R-VB-Out3** port. The buffer used in the chase is either conditioning buffer (**T-VB-In2**) when no diafiltration step has been included, or diafiltration buffer (**T-VB-In3** or **T-VB-In4**, according to the last diafiltration step before recovery).

Buffer flush after recovery

If the retentate is recovered, this can be followed by a defined number of flushes. The retaining product will be emptied through **R-VB-Out1**. To do this, use the following procedure:

Step	Action
1	Check the Buffer flushes collected through **R-VB-Out1** box.
2	Select number of flushes. Up to two flushes can be selected.
3	Enter the volume for each flush.



Note:

The minimum flush volume represents the buffer volume that is present in the recirculation loop after the buffer chase for primary recovery, and depends on the hold-up volume of the system.

4	To perform recirculation, select the ***Recirculation before recovery*** option. Recirculation can also be performed between buffer flushes. ***Note:*** *The product will leave the system through* **R-VB-Out3** *and the flush volumes will leave through* **R-VB-Out1**.

7.2 Microfiltration

About this section

This section provides information on how to create product methods for Microfiltration using the ***Method Wizard***.

In this section

7.2.1 Introduction

Depending on the application, the product of interest will either stay in the retentate or pass through the filter to the permeate side.

Cell harvesting/washing

In cell harvesting and cell washing, the product of interest (the cells) will stay in the retentate.

- A concentration step reduces the volume of the cell solution.
- A diafiltration step is run to wash the harvested cells.

Cell or lysate clarification

After cell harvesting, mechanical disruption of the cells releases the product of interest from the cells and creates a lysate.

In a concentration step, cells, cell debris or other insoluble matter are retained by the filter and the target product passes through the filter to the permeate.

Note: *In this step, the product leaves the system through* **P-VB-Out2**.

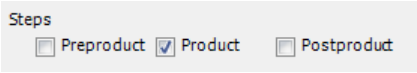
A diafiltration step is then performed to flush the rest of the product of interest through the membrane.

Note: *In this step, the product leaves the system through* **P-VB-Out3**.

7.2.2 Basic settings dialog

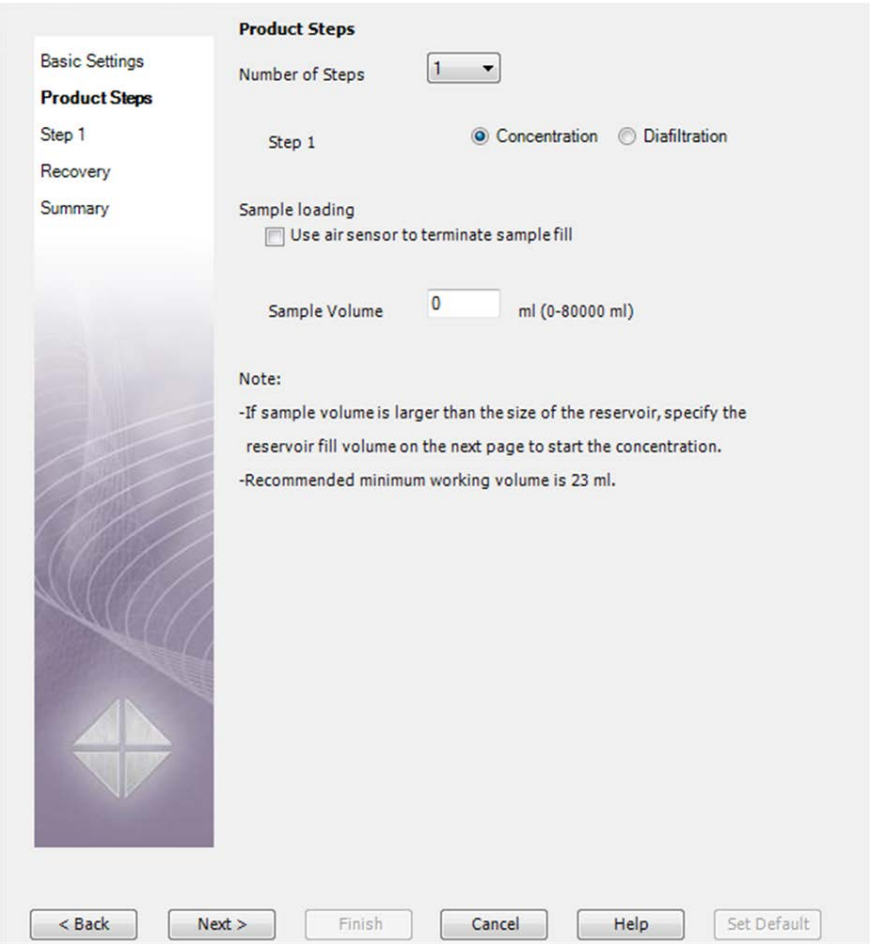
Input the basic settings (filter type, specifications, tubing kit i.d., and size of reservoir) as detailed in *Section 6.3.1 Basic settings dialog, on page 100*.

Select ***Product*** in the ***Steps*** selection.



7.2.3 Product steps dialog

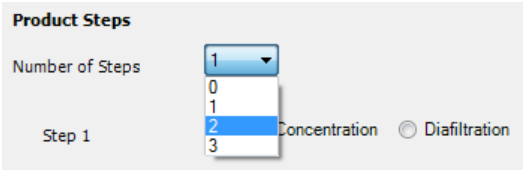
The image below shows the ***Product Steps*** dialog window.



A minimum working volume is displayed. The minimum working volume is the system hold-up volume including the filter hold-up, with an addition of a small volume in the reservoir. This volume depends on the reservoir size, tubing kit, and filter volume. This is the lowest working volume that is recommended.

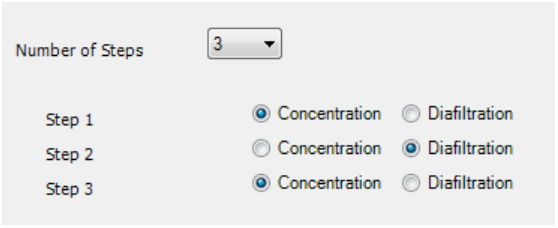
Note: *Minimum working volume will vary with reservoir size and tubing kit used and is not the same as system hold-up volume. To maintain smooth processing, a small volume is added to the system hold-up volume in the calculation of minimum working volume. For information on system hold-up volume, see the Appendix A.*

To enter information in to the ***Product Steps*** dialog, use the following procedure:

Step	Action
1	In the ***Product Steps*** dialog, select up to 3 concentration and diafiltration steps. 

Note:

For diafiltration, only 2 steps can be selected with any combination of steps. This is due to the limited number of transfer valve block inlets that are used as standard for diafiltration buffer.

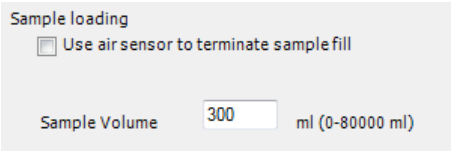


Step	Action
2	Enter the sample volume. There are 3 options:

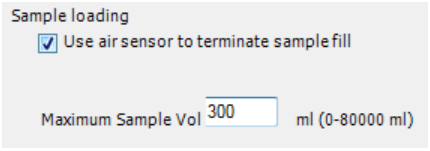
a. Enter a set volume.

Note:

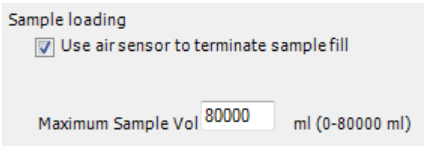
If a volume greater than the maximum volume of the chosen reservoir is entered, on the next page you will be required to enter a ***Fill Volume*** *and the system will operate in* ***Fed Batch*** *mode. In this case, the reservoir is continually refilled as a function of the permeate flow. This is possible when only concentration is the first step. Otherwise, if the total sample volume fits into the chosen reservoir size, the system will operate in* ***Tank Batch*** *mode.*



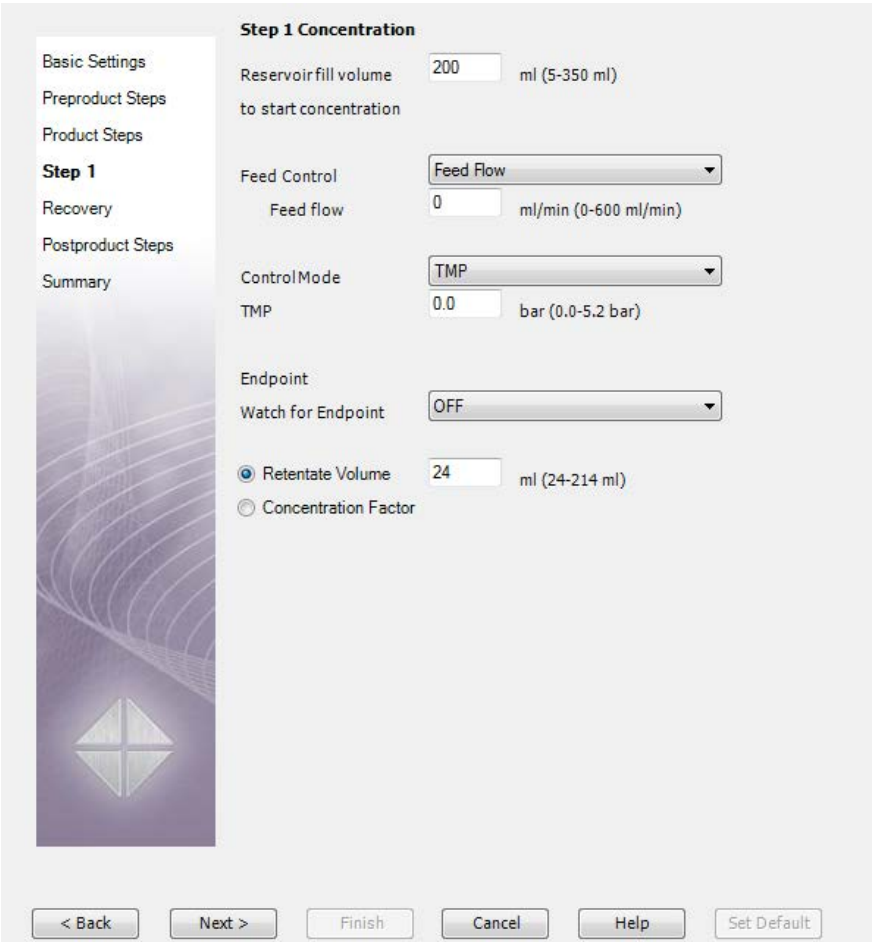
b. Enter a set volume and check ***Use air sensor to terminate sample fill***. This will activate the air sensor found on **T-VB-In1**. if air is detected before the sample volume is reached, the air sensor terminates the sample load, but if no air is detected before the sample volume is reached, the sample volume terminates the load.



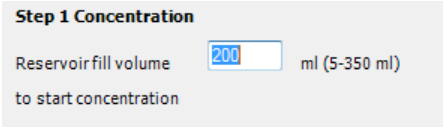
c. Check ***Use air sensor to terminate sample fill*** but leave the sample volume on the default of 80 000 mL. In this case, only air detection on **T-VB-In1** will terminate the sample load.



7.2.4 Concentration step dialog



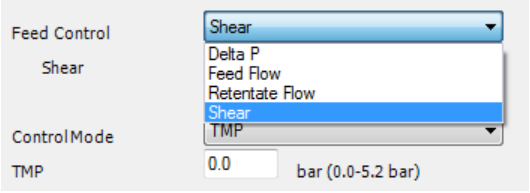
If a sample volume greater than the chosen reservoir volume has been selected for fed batch operation, or if the ***Use air sensor to terminate sample fill*** option has been selected, a reservoir fill volume must first be entered. The default fill volume is 200 mL for either reservoir but the maximum fill volume will depend on the size of the reservoir chosen. A reservoir minimum is also displayed; this is the minimum recommended working volume for the reservoir (5 mL for the small reservoir, 8 mL for the large reservoir).



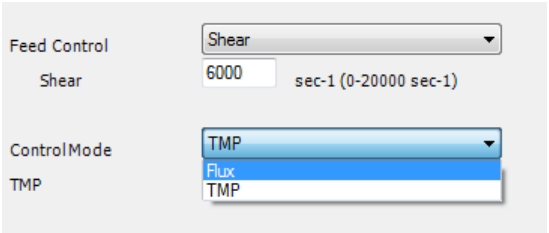
Feed and filtration control

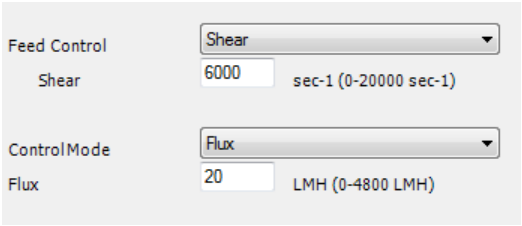
There are four ways to create the crossflow, all controlled by the feed pump:

- constant ***DeltaP***
- constant ***Feed Flow***
- constant ***Retentate Flow***
- ***Shear*** rate (only with hollow fibers)



To set the feed control settings, use the following procedure.

Step	Action
1	Enter the value for the selected crossflow rate, for example a ***Shear*** of 6000 s^{-1}. ***Note:*** *When using shear rate as crossflow rate for hollow fibers, the feed flow rate that equals a chosen shear rate must be calculated in advance; the maximum feed flow rate on the system is 600 mL/min, and if a shear rate for the selected hollow fiber exceeds this, the feed pump will run at 600 mL/min. No error or alarm will be generated. For more information on shear rates, and conversion to feed flow rate for a hollow fiber, refer to the Hollow Fiber Operating Guide.*
2	From the ***ControlMode*** drop down menu, select one of two available filtration control modes: ***TMP*** control or permeate ***flux*** control. ***Note:*** *Permeate flux control is recommended for any pore size over 0.1 µm, to prevent rapid blocking of the membrane. Low TMP control can often be used for high molecular weight ultrafilters (such as 500 or 750 kD NMWCO).* 

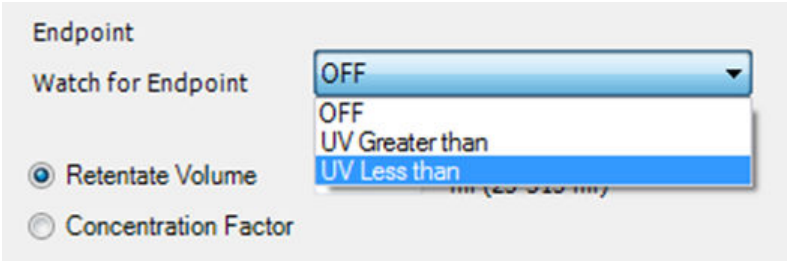
Step	Action
3	Enter the desired flux control value in LMH or TMP control value in bar.  ***Note:*** *When using TMP as the filtration control mode in a method, always choose a TMP value in the product steps that is below the TMP limit. Otherwise, the TMP limit may lead to a TMP pressure alarm, which will pause the run.*

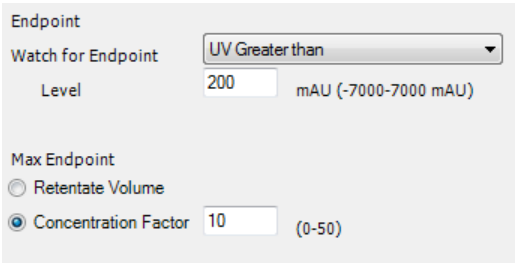
Concentration endpoint

There are two ways to end a concentration step:

- ***Watch for Endpoint***
- ***Retentate Volume/Concentration Factor***

To place a watch on the UV to end the concentration step, use the following procedure:

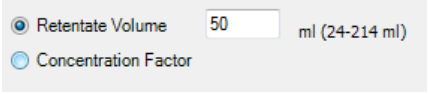
Step	Action
1	Select ***UV Greater than*** or ***UV Less than*** from the drop-down list. 

Step	Action
2	Enter an endpoint value in the ***Level*** window. UNICORN will end the concentration step based on the specified UV signal (in mAU) in the permeate. ***Note:*** *If the **Watch for Endpoint** setting is used, a maximum endpoint must still be designated. UNICORN will end the concentration step when either the UV level or maximum endpoint condition is met.*
3	Set a maximum endpoint value for either ***Retentate Volume*** or ***Concentration Factor***. 

Step	Action
1	Set the ***Watch for Endpoint*** option to ***OFF***.
2	Select either ***Retentate Volume*** or ***Concentration Factor*** under ***Max endpoint***.

Step	Action
3	Enter the value for the endpoint:

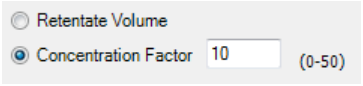
a. If a ***Retentate Volume*** is the desired endpoint, an applicable range will be given which depends on the total retentate hold-up volume, the fill volume, and the reservoir size.



Note:

It is not possible to end a concentration on a volume less than the fill volume plus the hold-up volume minus 5 mL. There is also a minimum retentate volume equal to the retentate hold-up volume plus 5 mL. This makes sure that a small amount of liquid remains in the reservoir at the end of the concentration step.

b. If a ***Concentration Factor*** is the desired endpoint, a concentration factor between 1 and 50 must be entered.



Note:

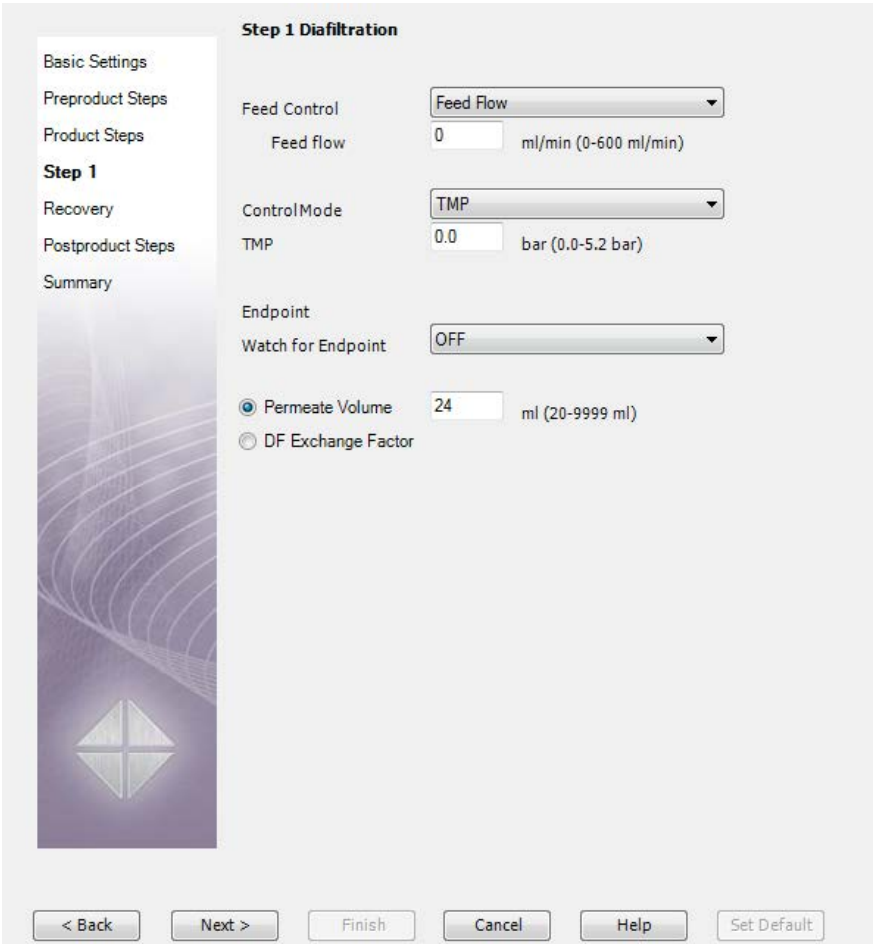
The expected sample volume and concentration factor must be estimated to make sure that they are achievable with the reservoir volume and retentate hold-up volume. For example, if you have an expected sample volume of 1000 mL, and are using the small reservoir, it is not possible to achieve a concentration factor of 2. The system will however not give an error message or alarm, but will end the concentration when the sample load reaches twice the fill volume plus the hold-up volume. Also, you may not be able to reach a desired concentration factor due to system minimum working volume limitation. For example, if the retentate hold-up volume is 25 mL, the sample volume is 100 mL, and the desired concentration factor is 5, the system's catastrophic ***ReservoirEmpty*** *alarm will pause the system when the level sensor detects an empty reservoir at 25 mL. It is recommended to use a minimum reservoir volume of 5 mL, although more may be required depending on crossflow rate.*

Note:

A concentration factor of 1 means no concentration, which can be used to compensate for an initial dilution due to liquid in the recirculation loop on the retentate side. This can be utilized in the diafiltration of small volumes as a planned single step. *See Diafiltration, on page 127.*

7.2.5 Diafiltration step dialog

If diafiltration of a small volume is planned, it is recommended to perform a concentration as an initial step. This is to avoid dilution of the sample due to the hold-up volume on the retentate side. Note also the sample volume limitation when performing a diafiltration as a first step. For more information, see *Diafiltration, on page 127* and *Concentration endpoint, on page 134*.



Feed and filtration control

See, *Section 7.2.4 Concentration step dialog, on page 150*.

Diafiltration endpoint

There are two ways to end a diafiltration step:

- ***Watch for Endpoint***
- ***Permeate Volume/Diafiltration Exchange Factor***

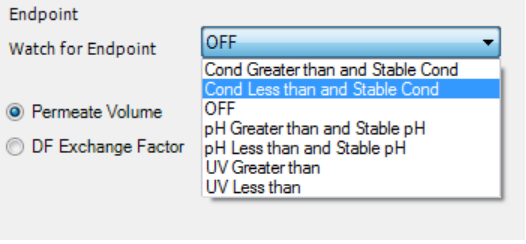
Three signals can be monitored to end a diafiltration step:

- conductivity
- pH
- UV

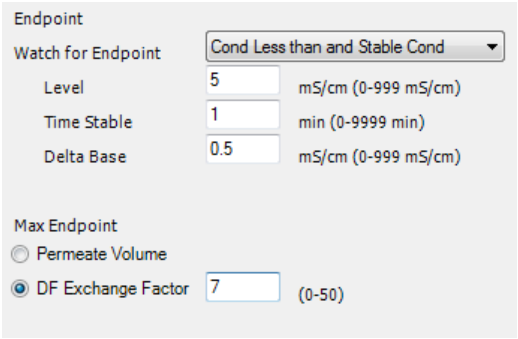
Note: *A maximum volume endpoint must be set using the **Max Endpoint** setting even if a **Watch for Endpoint** condition has been set. UNICORN will end the concentration step when either the monitor condition or maximum endpoint condition is met.*

Watch for endpoint

To place a watch to end the diafiltration step, use the following procedure:

Step	Action
1	In the ***Watch for Endpoint*** drop-down menu, select the signal and condition from the following options: **a.** ***Conductivity Greater than and Stable Conductivity*** **b.** ***Conductivity Less than and Stable Conductivity*** **c.** ***pH Geater than and Stable pH*** **d.** ***pH Less than and Stable pH*** **e.** ***UV Greater than*** **f.** ***UV Less than*** 
2	Enter the ***Level*** at which the monitoring of the stable signal should start.
3	Enter the ***Time Stable***, the time the signal should be stable for the signal watch to be met (conductivity or pH).

Step	Action
4	Enter ***Delta Base***, the allowed fluctuation (+ and – value) for the signal to be considered stable.



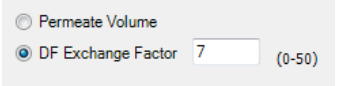
Note: *To end the diafiltration with a watch on the conductivity, pH, or UV signal, a maximum volume endpoint must still be designated. UNICORN will end the diafiltration step when either the monitor condition or maximum endpoint condition is met.*

Permeate Volume/Diafiltration Exchange Factor

To end the diafiltration step based on either a permeate volume or a diafiltration exchange factor, use the following procedure:

Step	Action
1	If ***Permeate Volume of DF Exchange Factor*** is to be the only endpoint then the ***Watch for Endpoint*** option must be set to ***OFF***.
2	Select either ***Permeate Volume*** or ***DF Exchange Factor*** and enter the endpoint value.

Note: *If a **Diafiltration Exchange Factor** is selected, a concentration factor between 1 and 50 must be entered.*



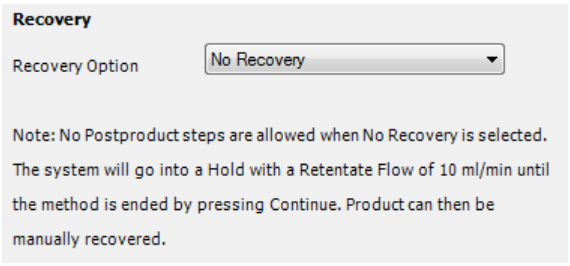
Note: *Due to possible unknown sample volume when loading based on air detection, UNICORN cannot estimate the required volume of diafiltration buffer. Make an approximate calculation to ensure that there is enough diafiltration buffer to achieve the desired diafiltration exchange factor. If the system runs out of diafiltration buffer, the sample will concentrate until the catastrophic* ***Reservoir Empty*** *alarm pauses the system.*

7.2.6 Recovery dialog

Recovery options

There are two options, ***Recovery*** or ***No Recovery***.

If ***No Recovery*** is selected, the system will go into a ***Hold*** with a retentate flow rate of 10 mL/min to avoid sedimentation. The material can either be recovered manually from the reservoir during the ***Hold***, or select ***Continue in System Control*** to end the method, allowing for manual recovery as desired.



Note: *If you opt for **No Recovery**, no postproduct steps are allowed*

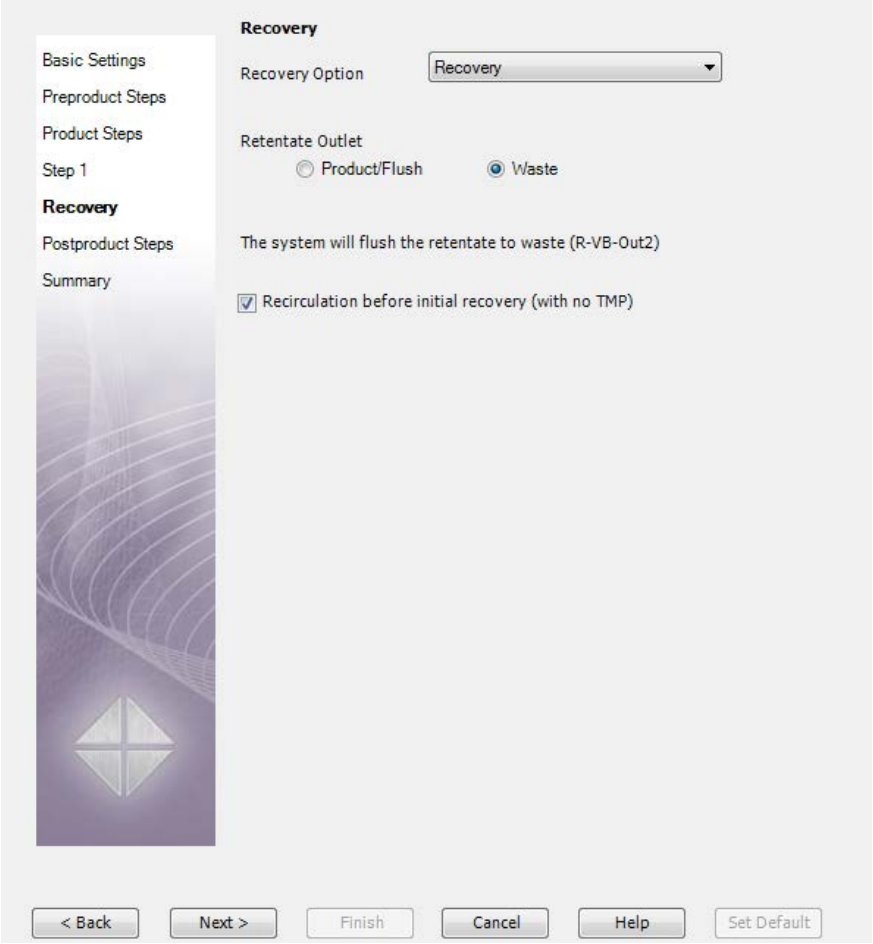
Note: *If an old type retentate valve is used on the system, be careful that any recovery vessels are not placed lower than the reservoir, as siphoning can occur.*

If ***Recovery*** is selected, the reservoir can be emptied by the following ways.

For flat sheet casettes, the reservoir is emptied through **R-VB-Out3**.

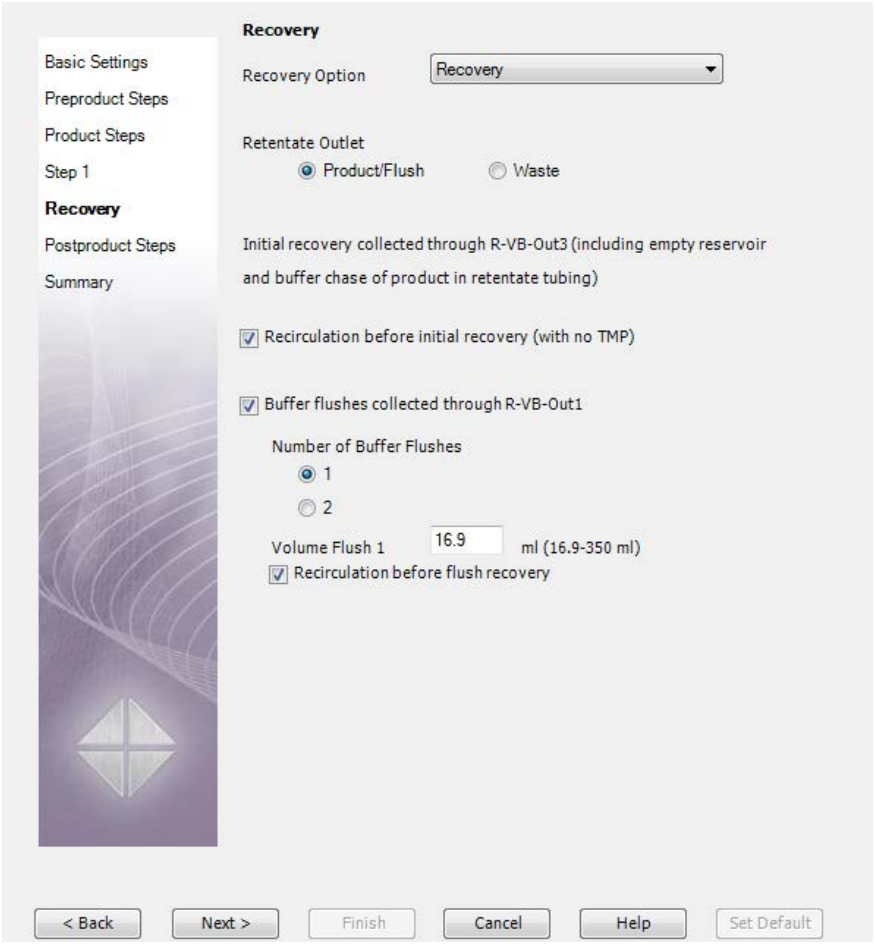
For hollow fibers, under ***Retentate Oulet*** select either:

- ***ProductFlush***: the initial recovery is collected through **R-VB-Out3** *or*
- ***Waste***: the retentate is sent to waste through **R-VB-Out2**



Note: *For clarification processes, the target product passes through the filter to the permeate. The retentate can be directed to waste. If waste is chosen, the product steps are terminated after an optional 5-minute recirculation with open TMP valve.*

If recovering the retentate with hollow fibers, select ***Product/Flush***.

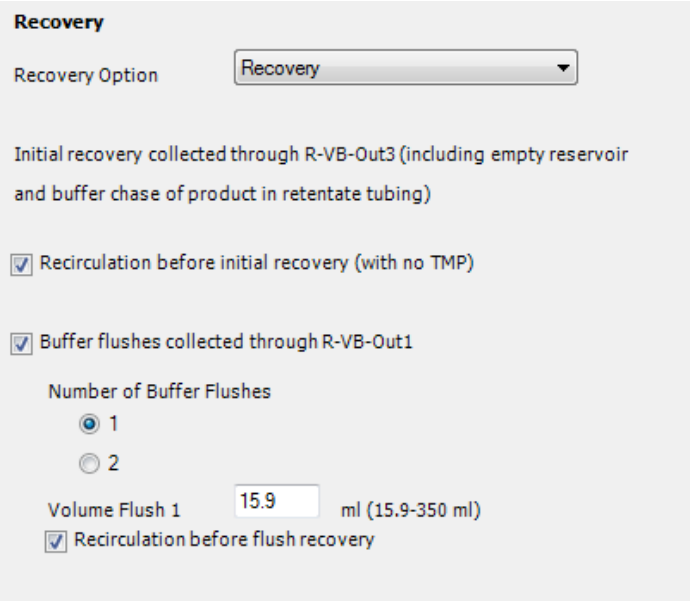


If ***Recirculation before initial recovery (with no TMP)*** is checked, the retentate is recirculated with an open **R-PCV** (TMP) valve for 5 minutes, to aid in the recovery process. This recirculation sweeps any proteins bound to the membrane (concentration polarisation) or gel layer that formed during the concentration and diafiltration process back into the bulk flow to enhance the yield. The retentate is then recovered through **R-VB-Out3**, followed by a buffer chase This allows recovery of the undiluted product in the reservoir and 70% of the undiluted material in the recirculation pathway to the **R-VB-Out3** port. The buffer used in the chase is either conditioning buffer (**T-VB-In2**) when no diafiltration step has been included, or diafiltration buffer (**T-VB-In3** or **T-VB-In4)**, according to the last diafiltration step before recovery.

Buffer flush after recovery

If the retentate is recovered, this can be followed by a defined number of flushes and the retaining product will be emptied through **R-VB-Out1**. To do this, use the following procedure:

Step	Action
1	Check the Buffer flushes collected through **R-VB-Out1** box.
2	Select number of flushes. Up to two flushes can be selected.
3	Enter the volume for each flush.



Note:

The minimum flush volume represents the buffer volume that is present in the recirculation loop after the buffer chase for primary recovery, and depends on the hold-up volume of the system.

4	To perform recirculation, select the ***Recirculation before recovery*** option. Recirculation can also be performed between buffer flushes. ***Note:*** *The product will leave the system through* **R-VB-Out3** *and the flush volumes will leave through* **R-VB-Out1**.

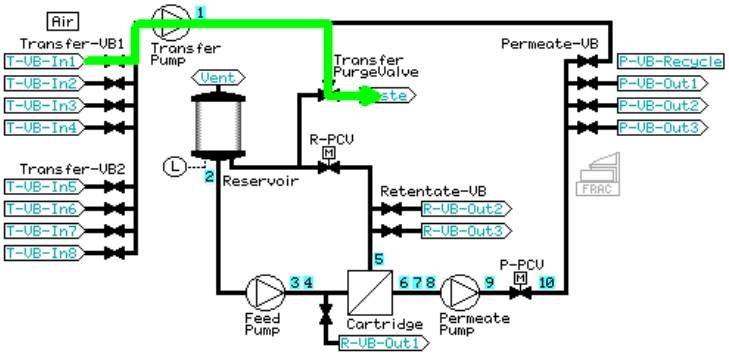
7.3 Visualization of product steps

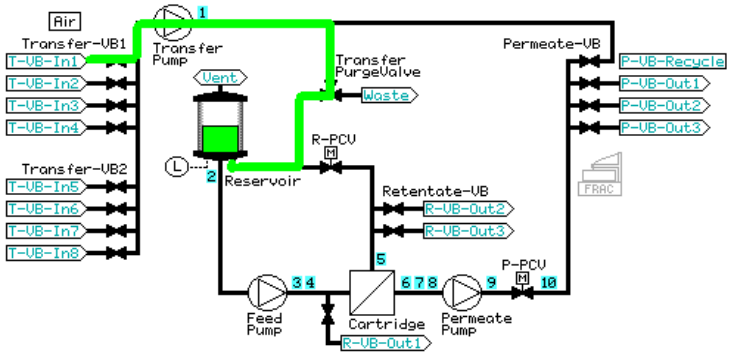
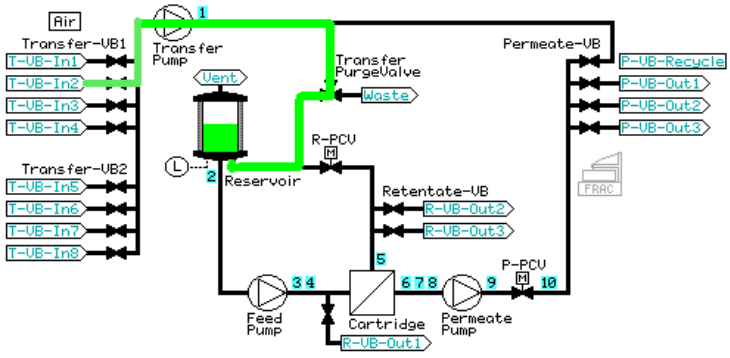
For information on the specific instructions, for example ***Constant Retentate Volume***, see *Chapter 14 Strategy instructions, on page 268*.

Sample filling

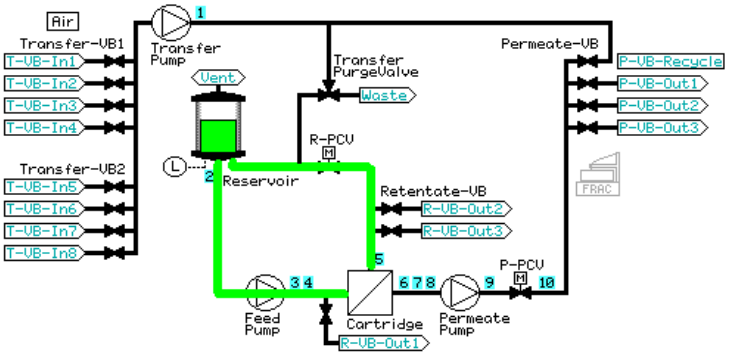
Sample filling, either **Tank Batch** (sample fill) or **Fed Batch** (reservoir fill) must take place before a concentration or diafiltration step.

Stage	Description
1	To prime the sample inlet tubing, the transfer pump first fills the tubing with 6 mL sample from **T-VB-In1** (sample).
2	To prime the tubing from the transfer pump to the transfer purge valve waste line, an additional 10 mL sample is filled into the tubing. Only the initial 6 mL priming volume goes to waste.

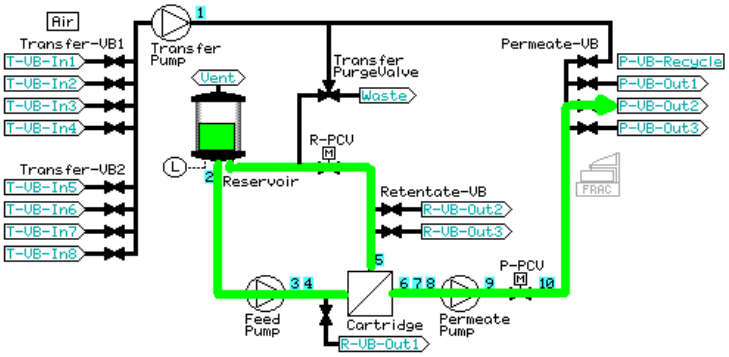


Stage	Description
3	The **Transfer_Purge_Valve** is switched to **Reservoir** to fill with sample. The volume which is initially filled depends on whether a ***Tank Batch*** or ***Fed Batch*** process is used. With a ***Tank Batch***, the total sample volume minus 30 mL is filled at a high flow rate, followed by an additional 20 mL at a slow flow rate (to improve accuracy). This is followed by the buffer chase detailed below, to bring in the remaining 10 mL sample contained within the tubing to the reservoir. 
4	The last 10 mL of the sample is chased with buffer (either conditioning or diafiltration buffer, depending on the product steps) to the reservoir. 
5	If the ***Use air sensor to terminate sample fill*** has been selected, the air sensor is activated during the sample load. If air is detected, the sample load is terminated early and the system moves to the next step.

Stage	Description
6	The sample is recirculated at the chosen crossflow rate until the deltaP has stabilized for at least 1 minute, with a timeout after 5 minutes.

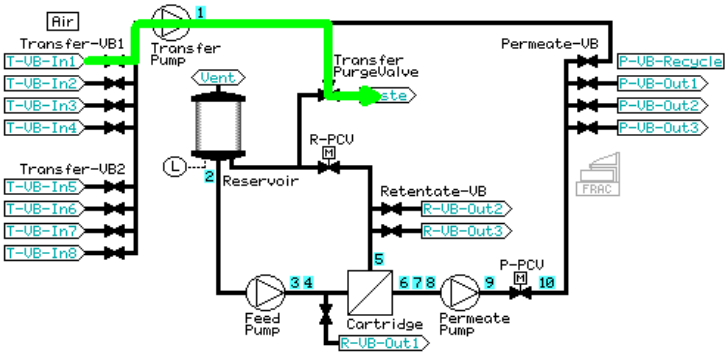


7	The appropriate permeate outlet is opened (**P-VB-Out2** for a concentration step or **P-VB-Out3** for a diafiltration step) and the filtration control mode is started.

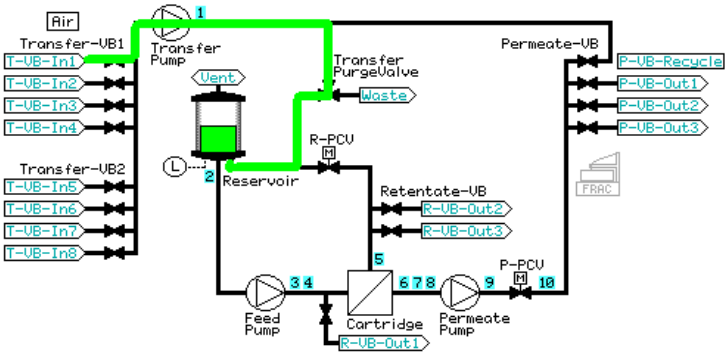


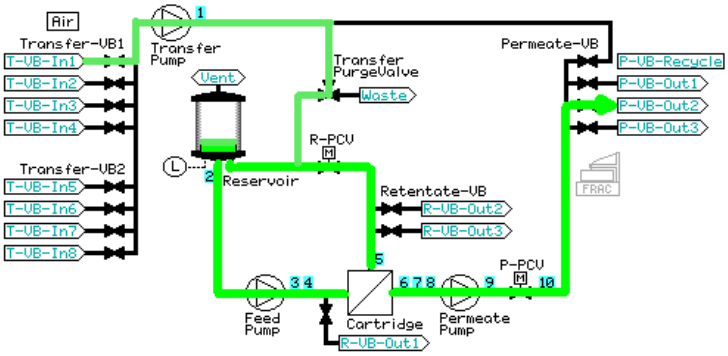
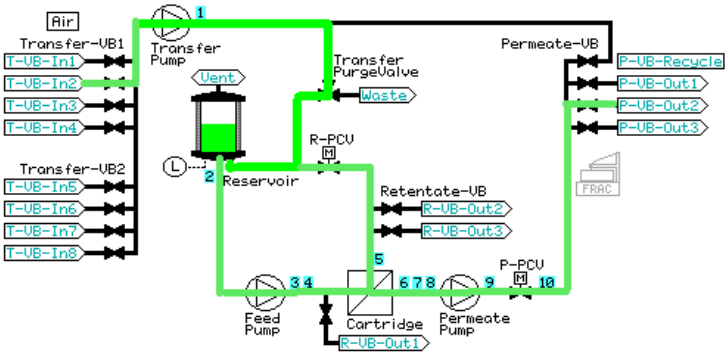
8	To prime the sample inlet tubing, the transfer pump first fills the tubing with 6 mL sample from **T-VB-In1** (sample).

Stage	Description
9	To prime the tubing from the transfer pump to the transfer purge valve waste line, an additional 10 mL sample is filled into the tubing. Only the initial 6 mL priming volume goes to waste.



10	With a ***Fed Batch*** process, the initial ***Reservoir fill volume*** minus 20 mL is filled at a high flow rate, followed by an additional 20 mL at a slow flow rate. The sample load will finish at the end of the ***Fed Batch*** with the buffer chase detailed below, to bring in the 10 mL sample contained within the tubing to the reservoir..

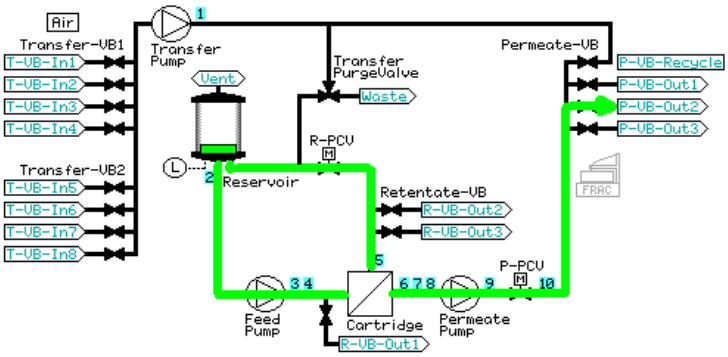


Stage	Description
11	***Constant retentate volume*** is activated. The selected crossflow is set, the permeate **P-VB-Out2** is opened, and the filtration control mode is started. 
12	A watch is put on the ***TransVol*** for the total sample volume minus 10 mL. When this is reached, the last 10 mL of the sample is chased with buffer (either conditioning or diafiltration buffer, depending on the product steps) to the reservoir. 
13	If the ***Use air sensor to terminate sample fill*** has been selected, the air sensor is activated during the sample load. If air is detected, the sample load is terminated early and the system moves to the next step.
14	The same principle is used with ***Use air sensor to terminate sample fill*** and unlimited sample volume; when air is detected, the sample load is terminated and the system moves to the next step.

Concentration

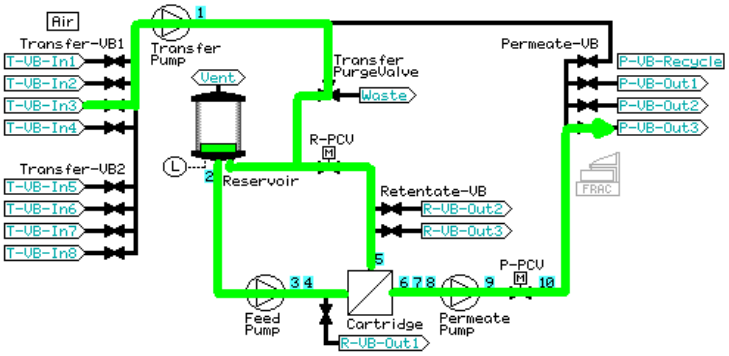
The concentration starts after the sample fill (**Tank Batch**) or reservoir fill (**Fed Batch**) has finished, with the opening of the permeate pathway **P-VB-Out2** and setting of the filtration control mode parameter.

Stage	Description
1	The concentration continues, with **P-VB-Out2** open, until the desired end point has been reached (either a specified concentration factor or retentate volume) or the watch on the UV signal has been met.



Diafiltration

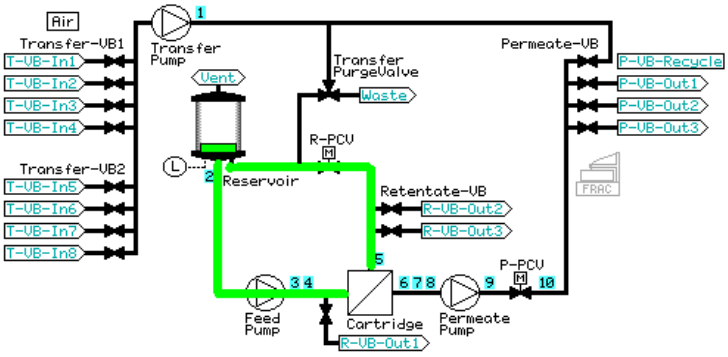
The diafiltration starts with a specific sample volume in the reservoir, either the specified end point of the concentration step, or the fill volume in a diafiltration-only method or method in which the diafiltration is the first step.

Stage	Description
1	*Constant Retentate Volume* is activated. The transfer valve inlet is opened to either diafiltration buffer 1 or 2 (depending on step) (**T-VB-In3** or **T-VB-In4**) and the permeate pathway is switched to **P-VB-Out3**. The crossflow rate and filtration control mode remain active or are changed (if specified in the method). 
2	Liquid leaving the system through **P-VB-Out3** is replaced with diafiltration buffer through **T-VB-In3** until the desired end point has been reached (diafiltration exchange factor or permeate volume) or a watch on the conductivity, pH, or UV signal has been met. If two diafiltration steps are performed, the second step will use buffer from **T-VB-In4**.

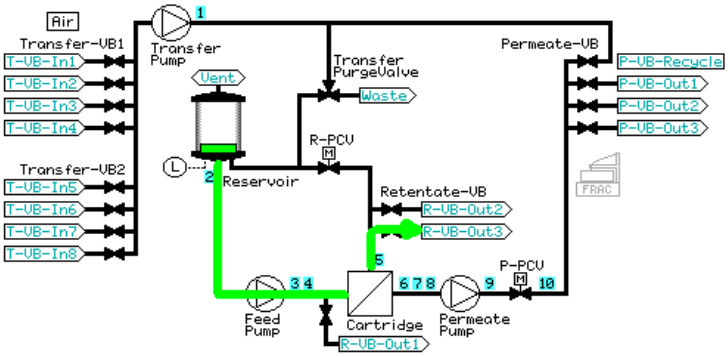
Recovery, no flush

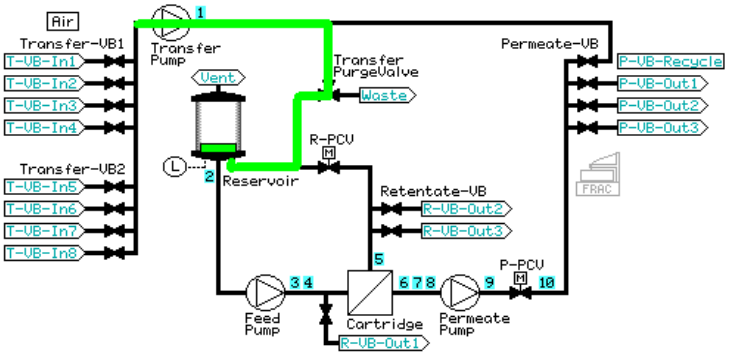
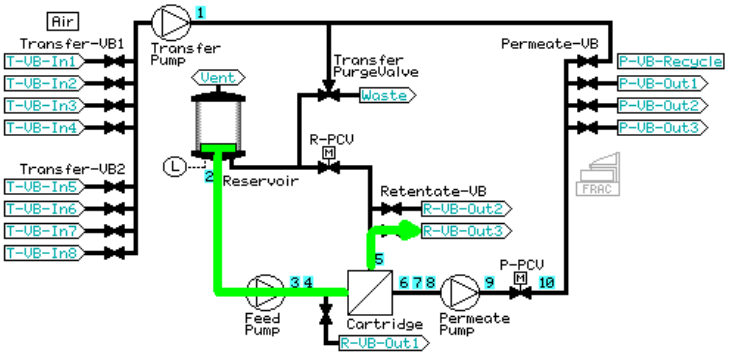
Product recovery without flush recovers cells from the ÄKTAcrossflow system.

Stage	Description
1	At the end of the final product step, the permeate pathway is closed and the filtration control mode de-activated. If ***Recirculation before initial recovery*** has been selected, the system will recirculate (using the same crossflow rate used in the last product step) for 5 minutes.



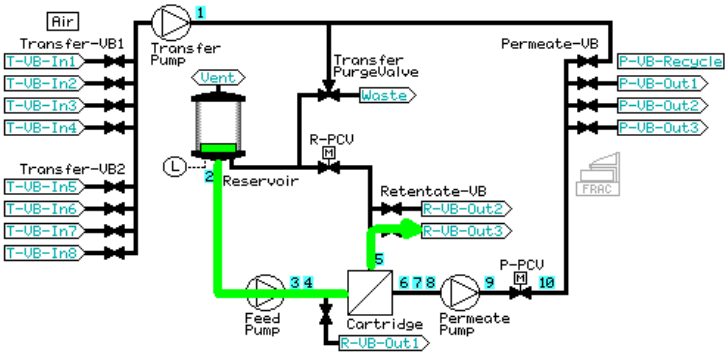
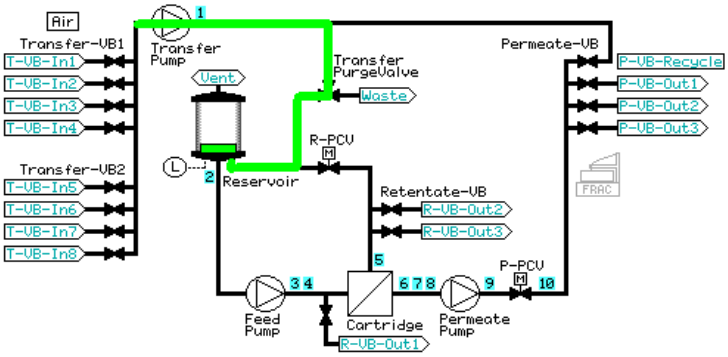
2	To start the recovery, the reservoir is emptied through **R-VB-Out3**.

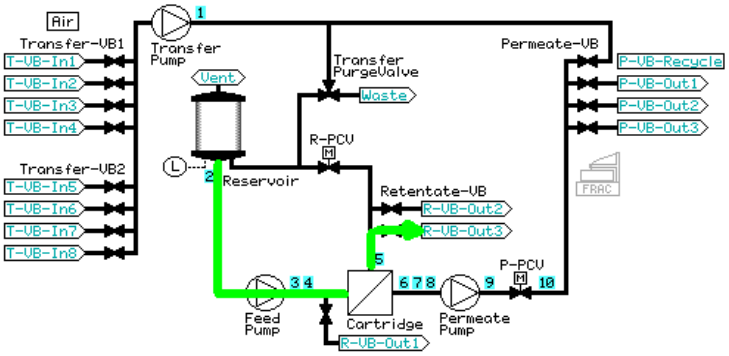
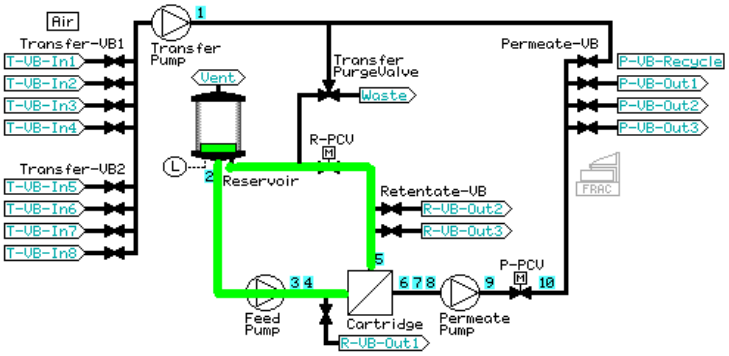


Stage	Description
3	The reservoir is then filled with 5 mL buffer (either conditioning from **T-VB-In2**, or diafiltration buffer from **T-VB-In3** or **T-VB-In4**, depending on the product steps chosen). 
4	At a transfer and retentate flow rate of 5 mL/min, buffer in the reservoir is used to chase 70% of the undiluted product in the pathway between the reservoir and **R-VB-Out3** out through **R-VB-Out3**, adding to the undiluted product recovered in the initial emptying of the reservoir. 

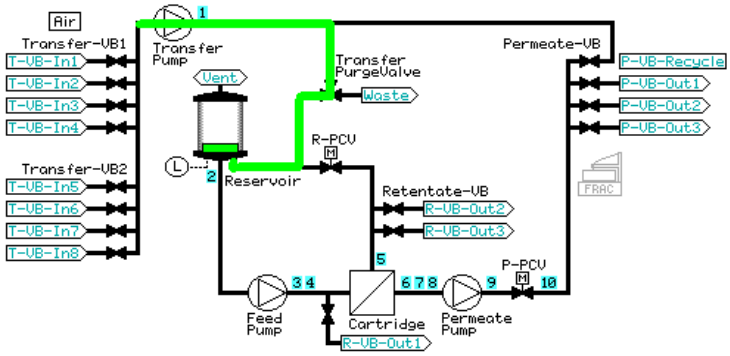
Recovery, with buffer flushes

The ***Recovery*** with buffer flushes removes residual product from system without risking precipitation of components on the membrane or flow path before product recovery.

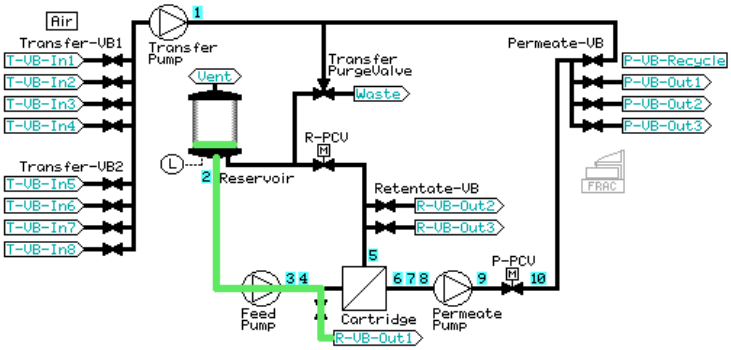
Stage	Description
1	At the end of the final product step, the permeate pathway is closed and the filtration control mode de-activated. If ***Recirculation before initial recovery*** has been selected, the system will recirculate (using the same crossflow rate used in the last product step) for 5 minutes.
2	To start the recovery, the reservoir is emptied through **R-VB-Out3**. 
3	The reservoir is filled with the volume of buffer specified in ***Volume Flush 1*** in the ***Recovery*** dialog. This buffer is either conditioning buffer from **T-VB-In2**, if no diafiltration step was chosen, or diafiltration buffer from **T-VB-In3** or **T-VB-In4**, depending on the last diafiltration step. 

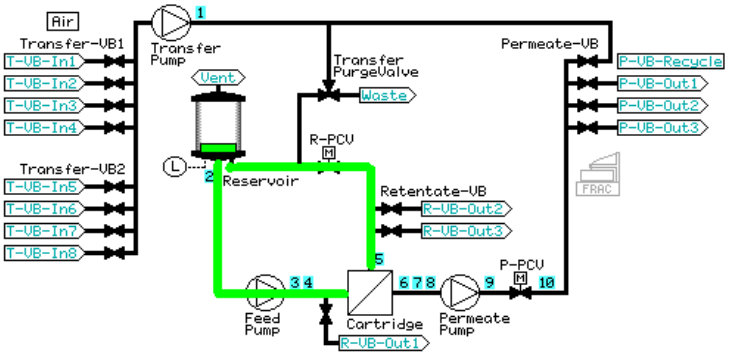
Stage	Description
4	At a transfer and retentate flow rate of 5 mL/min, buffer in the reservoir is used to chase 70% of the undiluted product in the pathway between the reservoir and **R-VB-Out3** out through **RVB-Out3**, adding to the undiluted product recovered in the initial emptying of the reservoir. 
5	**R-VB-Out3** is closed and the retentate valve block is set to **R-VB-Recycle**. If ***Recirculate before flush recovery*** has been selected, the retentate is recirculated for five minutes at the same crossflow rate chosen for the last product step. 
6	**R-VB-Out1** is then opened, and the reservoir is emptied.

Stage	Description
7	If a second buffer flush has been selected, the reservoir is then filled with the volume of buffer specified in ***Volume Flush 2*** in the ***Recovery*** dialog. This buffer is either conditioning buffer from **T-VB-In2**, if no diafiltration step was chosen, or diafiltration buffer from **T-VB-In3** or **T-VB-In4**, depending on the last diafiltration step.



8	At a transfer and retentate flow rate of 5 mL/min, buffer in the reservoir is used to chase 70% of the dilute product in the pathway between the reservoir and **R-VB-Out1** out through **R-VB-Out1**, adding to the first flush recovered in the emptying of the reservoir out **R-VB-Out1**.



Stage	Description
9	**R-VB-Out3** is closed and the retentate valve block is set to recycle. If ***Recirculate before flush recovery*** has been selected, the retentate is recirculated for five minutes at the same crossflow rate chosen for the last product step.*Result:* **R-VB-Out1** is then opened, and the reservoir is emptied. 
10	At a transfer and retentate flow rate of 5 mL/min, buffer in the reservoir is used to chase 70% of the dilute product in the pathway between the reservoir and **R-VB-Out1** out through **R-VB-Out1**, adding to the dilute product recovered in the initial emptying of the reservoir out **RVB-Out1**.
11	The rest of the reservoir volume is emptied through waste (**R-VB-Out2**).

8 Create postproduct steps using the Method Wizard

About this chapter

This chapter describes the preproduct steps and how to create them in the ***Method Wizard***.

In this chapter

8.1 Introduction

The ÄKTAcrossflow Method Wizard enables the simple creation of a series of steps to be used after a process run. These steps include:

- ***Flush***
- ***Clean-in-place***
- ***Water Flush***
- ***Water Flux Test***
- ***Filter Storage***

Note: *A recommended storage solution for the system is 20% ethanol. The system should not be stored in NaOH, as the pump seals are affected over time.*

Note: *To completely exchange the solution in the system, perform the* ***System Sanitization*** *method and use 20% ethanol instead of NaOH. It is important to use a three-way-connector instead of a filter. See Section 11.1 System sanitization, on page 218 for more information.*

Note: *If the system requires more intensive cleaning, it may also be necessary to perform a System Sanitization. See Section 11.1 System sanitization, on page 218.*

8.2 Postproduct steps: Description

Flush

After a product run, this step flushes remaining product and contaminants out of the filter before the CIP step. Select flush solution (either conditioning buffer from **T-VB-In2** or water from **T-VB-In5**). The flush volume is editable with a default value of 2 mL per cm^2 surface area of the filter. This step will:

- Prime the selected transfer inlet valve position (**T-VB-In2** or **T-VB-In5**) to waste
- Fill the reservoir to the maximum volume (350 mL for the small reservoir and 1100 mL for the large reservoir), then empty the reservoir out **R-VB-Out2**
- Flush the recirculation loop twice and empty the reservoir
- Refill the reservoir to a small volume and flush 2 mL per cm^2 surface area of the filter out through permeate valve block position 1 (**P-VB-Out1**)
- Empty the reservoir

Filter CIP

The filter CIP postproduct step includes an option to perform two filter CIP procedures with an optional water flush between. This step will:

- Prime the CIP transfer inlet valve positions tubing to waste.

  Note: *If CIP 1 only is chosen, only transfer valve block 6 (***T-VB-In6***) is primed. If CIP 2 only is chosen, only transfer valve block 7 (***T-VB-In7***) is primed. If both CIP 1 and CIP 2 are selected, both inlets will be primed.*

- Fill the reservoir to the maximum volume (350 mL for the small reservoir and 1100 mL for the large reservoir), then empty the reservoir out **R-VB-Out2**
- Flush the recirculation loop twice and empty the reservoir
- Either fill the reservoir to the maximum volume (small reservoir) or fill to a specified fill volume (large reservoir)
- Rinse 30 mL CIP solution to waste through **P-VB-Out1**
- Recirculate the permeate back into the reservoir for the ***Length of Time*** specified in the dialog
- Empty the reservoir

Empty the reservoir

If a water flush is chosen between CIP 1 and CIP 2, the system will:

- Prime the transfer inlet valve position 5 (**T-VB-In5**) tubing to waste

- Add a small volume of water to the reservoir to rinse the retentate loop
- Empty the reservoir
- Refill the reservoir to a small volume and flush 10 mL water out through the permeate valve block position recycle (**P-VB-Recycle**) to waste (**Transfer_Purge_Valve** to **Waste**)
- Empty the reservoir

If CIP 3 is chosen, this step will:

- Prime the transfer inlet valve position 7 (**T-VB-In7**) tubing to waste
- Add CIP solution to the reservoir to rinse the retentate loop
- Empty the reservoir
- Either fill the reservoir to the maximum volume (small reservoir) or fill to a specified fill volume (large reservoir)
- Rinse 30 mL CIP solution to waste through **P-VB-Out1**
- Recirculate the permeate back into the reservoir for the ***Length of Time*** specified in the dialog
- Empty the reservoir

Note: *The system will not be sanitized. For sanitization of the system, see Section 11.1 System sanitization, on page 218.*

Note: *If only a CIP 2 step is chosen, the procedure used by the system will be the same as the CIP 1 step, using transfer inlet position 7 (***T-VB-In7***) instead of transfer inlet position 6 (***T-VB-In6***).*

Water flush

The postproduct water flush step will fill the reservoir to the maximum volume (350 mL for the small reservoir and 1100 mL for the large reservoir) and empty, to make sure that any previous solution has been removed. It is always recommended to select a water flush after a filter CIP step where NaOH was used. This step should also be used before a water flux test is performed. The default water flush volume is 2 mL per cm^2 surface area of the filter and can be edited. This step will:

- Prime the transfer inlet valve position 5 (**T-VB-In5**) tubing to waste
- Fill the reservoir to maximum volume (350 mL for the small reservoir and 1100 mL for the large reservoir) and empty
- Add a small volume of water to the reservoir to rinse the retentate loop twice
- Empty the reservoir
- Refill the reservoir to a small volume and flush 2 mL water per cm^2 surface area of the filter out through permeate valve block position 1 (**P-VB-Out1**)

- Empty the reservoir

Water flux test

A water flux test measures the water permeability of a filter, to control the quality status of the filter. Permeate flux values are often normalized to 1 bar transmembrane pressure and corrected to a temperature of 25°C, and is then called the normalized water permeability (NWP; also called normalized water flux, or NWF). By comparing obtained water fluxes as a function of usage over time, it is possible to assess the efficiency of the filter CIP determine the lifetime of a filter.

It is recommended to perform the test before a product step, and after a product step and filter cleaning.

Always perform a water flush or rinse before a water flux test, to make sure that the filter is thoroughly flushed with water.

The filtration control mode is dependent on the filter type used.

- For flat sheet cassettes, TMP control mode is used. The default TMP value is 1 bar, but this can be edited by the user.
- For ultrafilter hollow fibers, TMP control mode is used. The default TMP value is 1 bar, but this can be edited by the user. It is recommended to set the TMP value to 0.5 bar or lower for high molecular weight hollow fiber ultrafilters (≥ 500 kD).
- For microfilter hollow fibers (cut off larger than 0.1 µm), ***Normal Flow Filtration*** (NFF) mode is used. ***Feed flow*** or ***Feed pressure*** can be selected as a feed control and a value is entered.

This step will:

- Prime the transfer inlet valve position 5 (**T-VB-In5**) tubing to waste
- Add a small volume of water to the reservoir to rinse the retentate loop
- Empty the reservoir
- Fill a small volume of water to the reservoir
- Set the system to total recycle (**P-VB-Recycle**, **Transfer_Purge_Valve** to **Reservoir**)
- Set the filtration control mode and wait until the flux has stabilized
- Measure the permeate flux and set a ***Set_Eval_Mark*** with the parameter ***Normalized_Water_Flux*** for easy analysis in the Evaluation module.

Data from water flux testing can be analyzed in the ***Evaluation module*** of UNICORN. In the example in *Figure 8.1, on page 181*, normalized water flux results from a series of measurements are plotted against the number of performed runs with a filter. A standardized temperature correction table compensates for temperature effects due to viscosity. Results are compared to previous tests and provide information about the quality status of the filter.

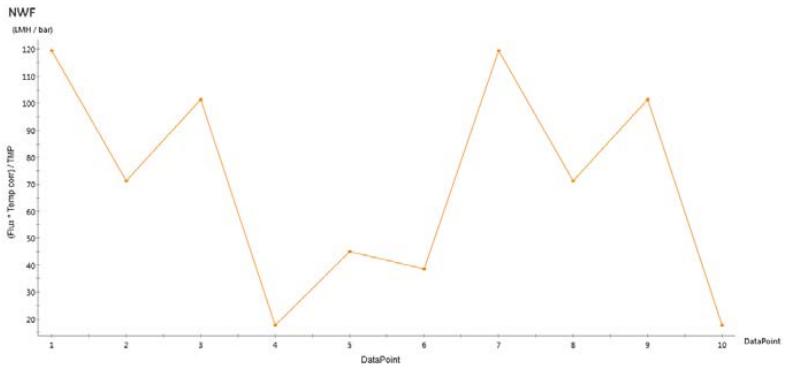


Figure 8.1: Example of plotted normalized water flux values

Note: *When comparing status of a filter as a function of time and number of uses, use the same filtration mode parameter each time the water flux test is run.*

Storage

The storage step replaces the liquid in the filter with a buffer that is suitable for storing the filter. This step will:

- Prime the transfer inlet valve position 8 (**T-VB-In8**) tubing to waste
- Add a small volume of storage solution to the reservoir to rinse the retentate loop
- Empty the reservoir
- Fill a small volume of storage solution to the reservoir
- Flush the filter with the selected volume, through permeate recycle to waste (**P-VB-Recycle**, transfer purge valve to waste)
- Set the system to total recycle (**P-VB-Recycle**, **Transfer_Purge_Valve** to **Reservoir**) for 5 minutes
- Empty the reservoir

Note: *See the filter manufacturer's instructions for suitable filter storage solutions. The system might require flushing after removing the filter to put in a suitable storage solution, such as 20% ethanol. Do not store the ÄKTAcrossflow in NaOH, as the pump seals may be affected.*

8.3 Postproduct steps: Method Wizard dialogs

About this section

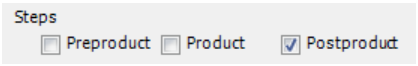
This section provides information on how to create postproduct steps in the ***Method Wizard*** when using hollow fibres and flat sheet cassettes, and provides a description of each step.

In this section

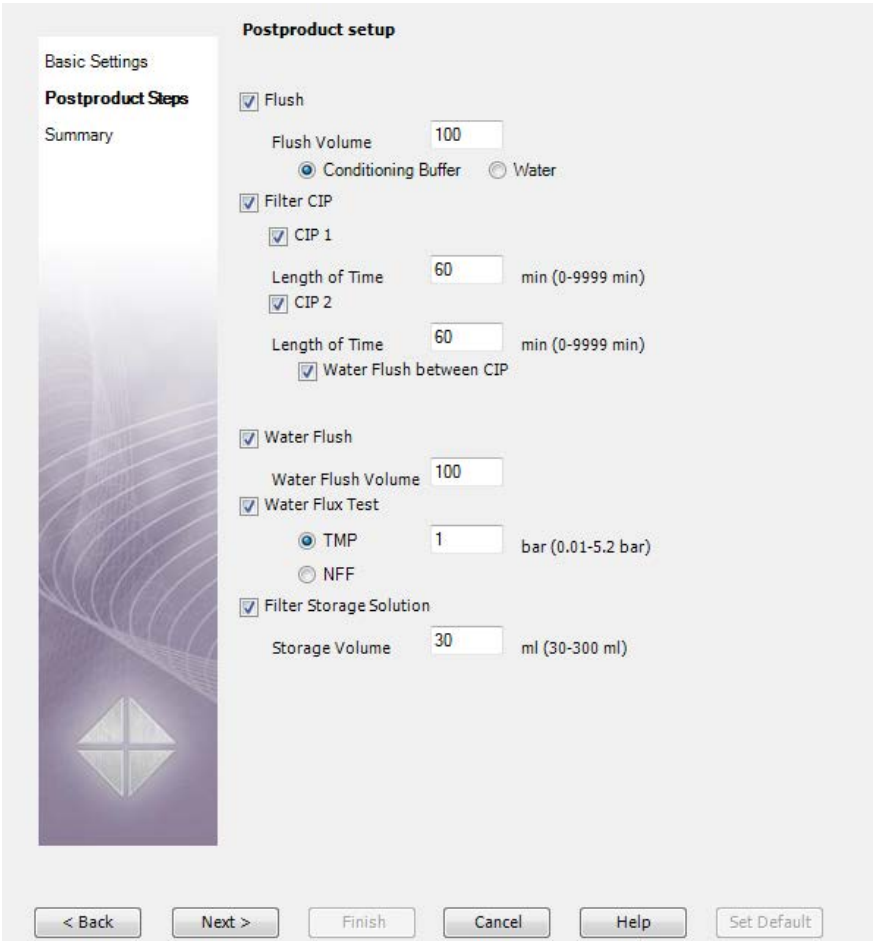
8.3.1 Postproduct step dialog

Postproduct step dialog overview

Input the basic settings (filter type, specifications, tubing kit i.d., and size of reservoir) as detailed in *Section 6.3 Preproduct steps: Method Wizard dialogs, on page 99*. Select ***Postproduct*** in the ***Steps*** selection.



The image below shows an example of the ***Postproduct setup*** dialog.

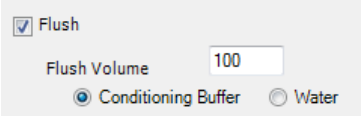


Flush

To include a conditioning buffer or water flush in the method, check the ***Flush*** box.

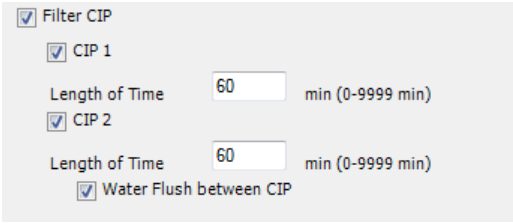
Select the flush volume; this is the volume of solution which will be flushed through the filter into the permeate. The default value is 2 mL per cm^2 of filter surface area.

Pick the flush solution: conditioning buffer (**T-VB-In2**) or water (**T-VB-In5**).



Filter CIP

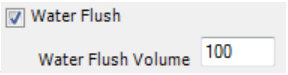
To include a Filter CIP step, use the following procedure.

Step	Action
1	Check the ***Filter CIP*** box.
2	Select either a one- or two-step CIP, with an optional water flush in between.
3	Enter the desired CIP circulation time in the ***Length of Time*** box. 

Water flush

To include a water flush step, use the following procedure.

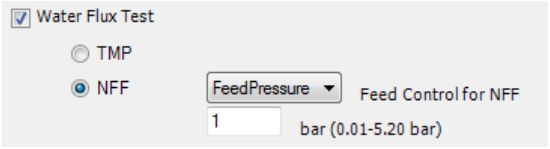
Step	Action
1	Check the ***Water Flush*** box.

Step	Action
2	Select the flush volume; this is the volume of water that will be flushed through the filter into the permeate. The default value is 2 mL per cm^2 of filter surface area.  **Note:** *After a filter CIP, running a water flush is recommended.*

Water flux test

To include a water flux test step, use the following procedure.

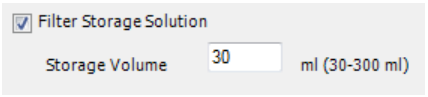
Note: *If a water flux test will be performed, a water flush should be performed just before the water flux test.*

Step	Action
1	Check the ***Water Flux Test*** box.
2	Select either ***TMP*** or ***NFF*** (***Normal Flow Filtration***) as the control mode.
3	Enter a value for the Flux Test: **a.** For TMP, a value of 1 bar is default for all ultrafilters; for high molecular weight hollow fiber ultrafilters (≥ 500 kD), it is recommended to set this value to 0.5 bar.  **b.** ***NFF*** (***Feed Pressure***) is default for microfiltration hollow fibers with a cut off of 0.1 µm and larger.  **c.** NFF control of feed flow is also possible.

Note: *When comparing status of a filter as a function of time and number of experiments, it is recommended to use the same filtration mode parameter each time the water flux test is run.*

Storage

To flush the filter with storage solution as part of the method, use the following procedure.

Step	Action
1	Check the ***Filter Storage Solution*** box.
2	Select the storage solution rinse volume; this is the volume of storage solution which will be flushed through the filter into the permeate. The default value is 30 mL, but this can be edited to a maximum value of 300 mL for the small reservoir or 1000 mL for the large reservoir. 

8.3.2 Visualization of the postproduct steps

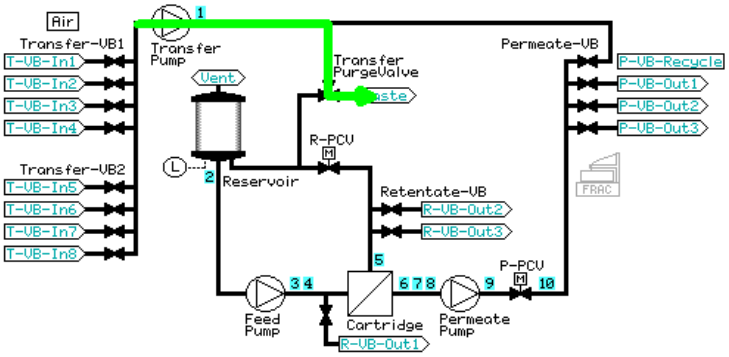
For information on specific instructions, for example ***Constant Retentate Volume***, *Chapter 14 Strategy instructions, on page 268*.

All postproduct steps begin with a ***Prepare System and Reservoir*** block, except for the ***Storage*** step and stand-alone ***Water Flux Test***, which begin with the ***Prepare System*** block. The ***Prepare System*** block is a quick flush of the recirculation loop, while the ***Prepare System and Reservoir*** step fills and empties the reservoir completely and thoroughly flushes the recirculation loop. The inlet valve position chosen depends on the liquid of the specific step.

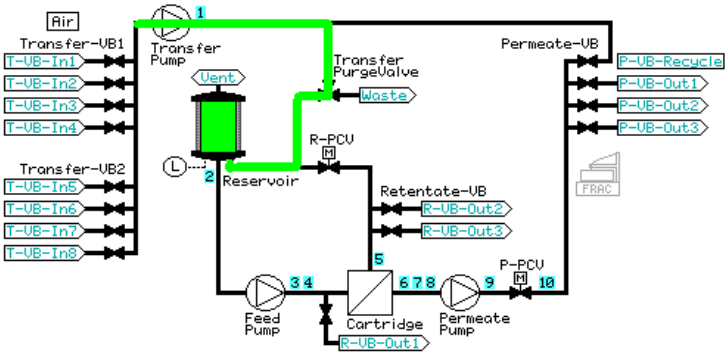
Prepare system and reservoir

The ***Prepare System and Reservoir*** step fills and empties the reservoir completely and thoroughly flushes the recirculation loop. The inlet valve position chosen depends on the liquid of the specific step.

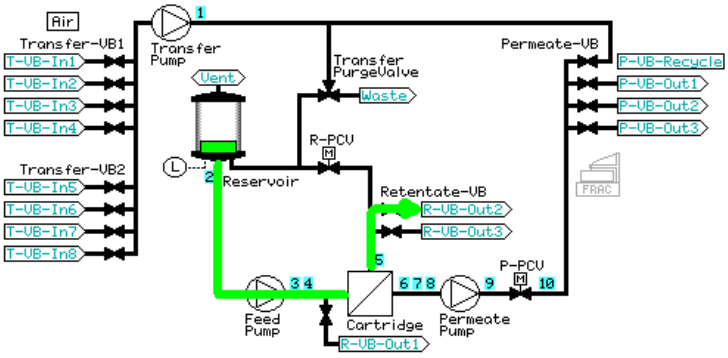
Stage	Description
1	The transfer inlet used is primed to waste through the transfer purge valve.



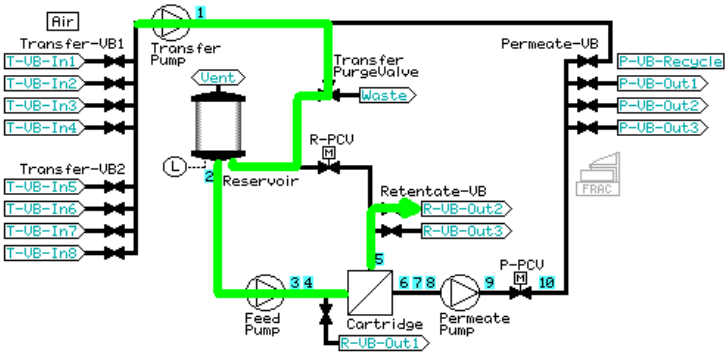
Stage	Description
2	The reservoir is then filled to the maximum volume (350 mL for the small reservoir, 1100 mL for the large reservoir).

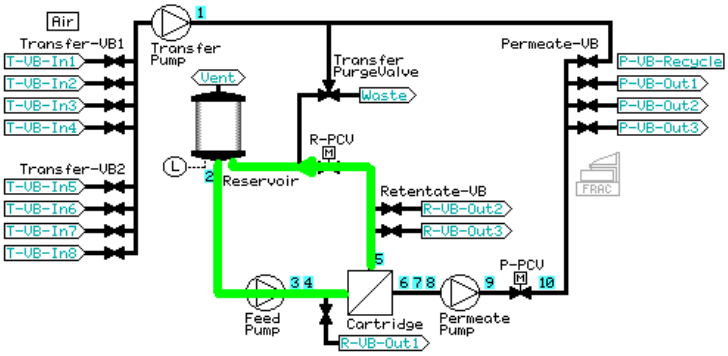
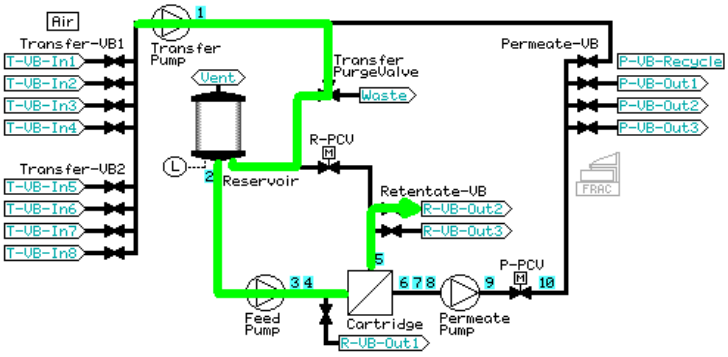


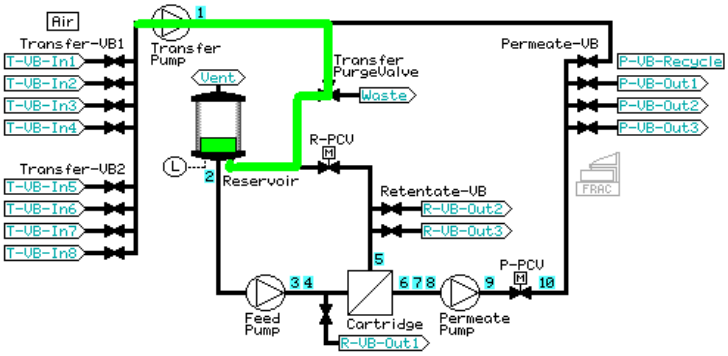
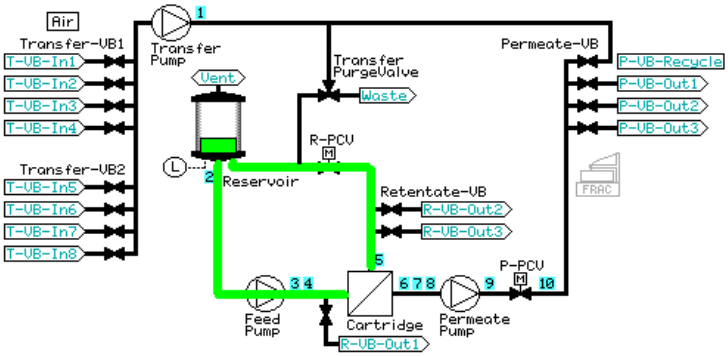
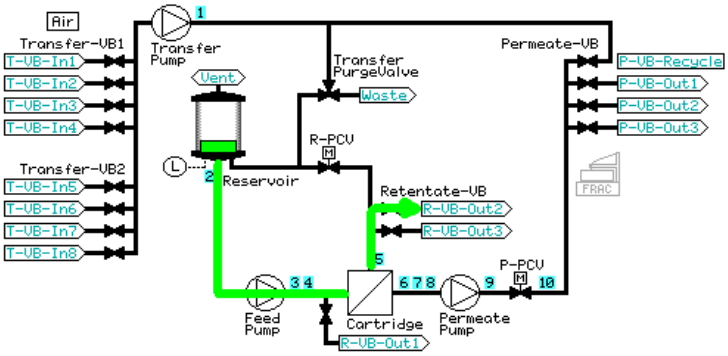
3	The reservoir is emptied through **R-VB-Out2** (waste).



4	The reservoir is filled to a minimum working volume. A low transfer and equal feed flow is set, and 50 mL is pumped out of the retentate to waste through **R-VB-Out2**.



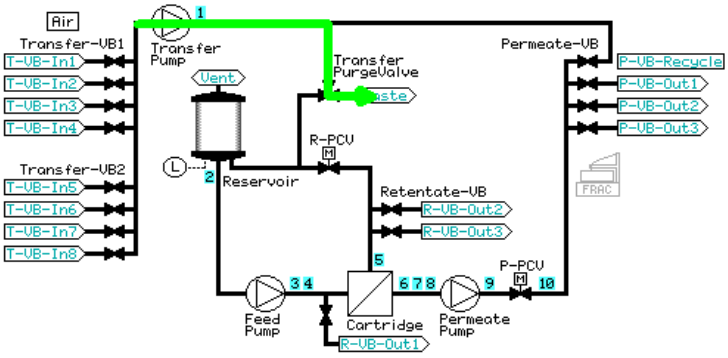
Stage	Description
5	The tubing in the pathway between **R-VB-Out2** and the reservoir is rinsed by recirculating at a low feed flow rate for less than a minute. 
6	The reservoir is emptied and refilled with the minimum working volume.
7	A low transfer and equal feed flow is set, and 50 mL is pumped out of the retentate to waste through **R-VB-Out2**. 

Stage	Description
8	The reservoir is filled to a low volume. 
9	The retentate is recirculated for one minute using a feed pressure control of 80% of the maximum feed pressure value (to a maximum of 3 bar). 
10	Then, the reservoir is emptied through **R-VB-Out2**. 

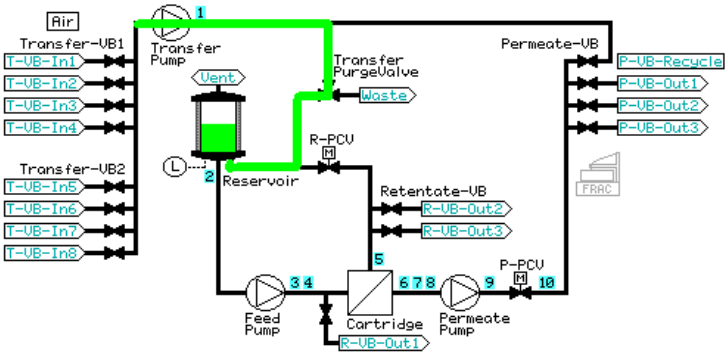
Prepare system

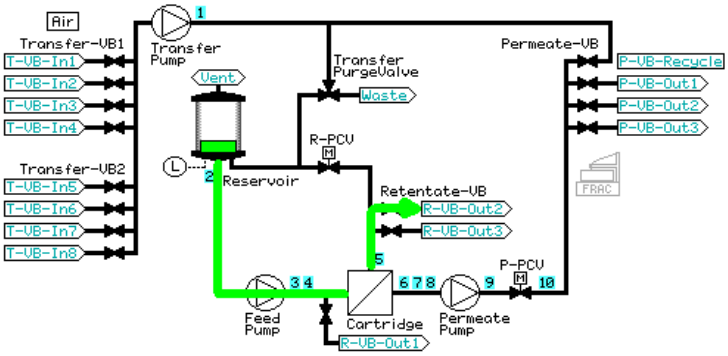
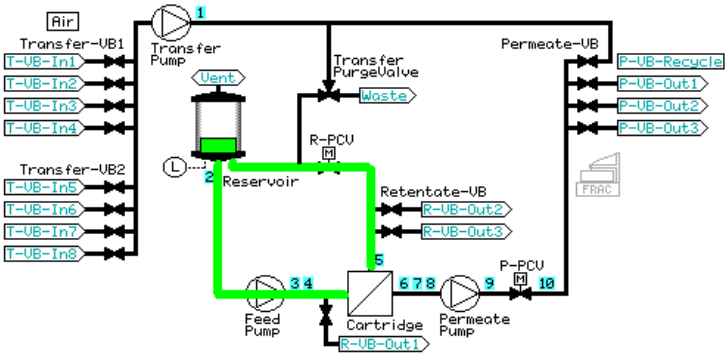
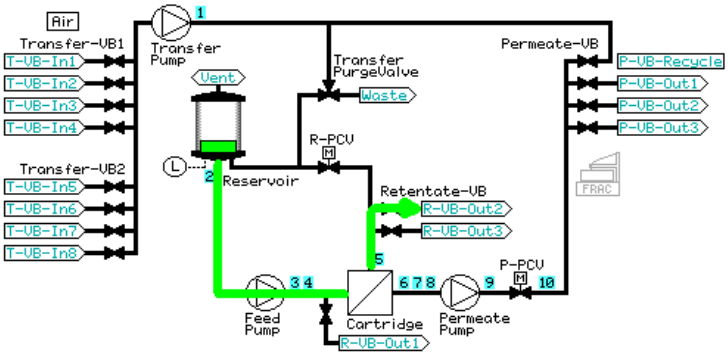
The ***Prepare System*** step performs a flush of the recirculation loop.

Stage	Description
1	The transfer inlet used is primed to waste through the transfer purge valve.



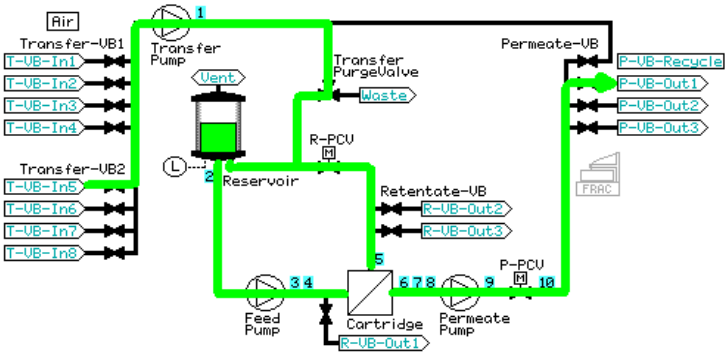
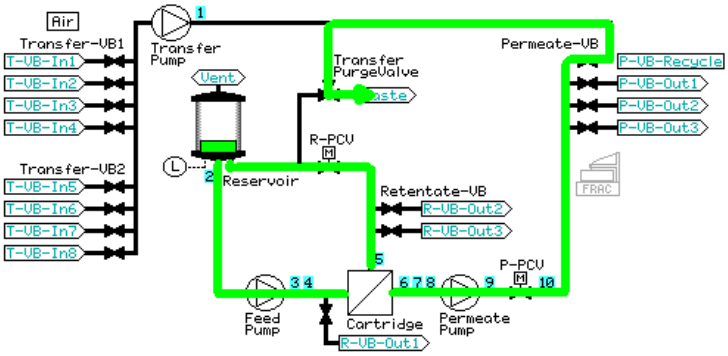
2	The reservoir is filled to a low volume.

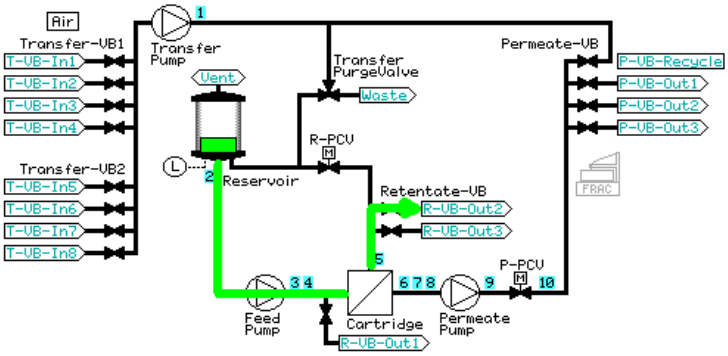


Stage	Description
3	The retentate is rinsed out **R-VB-Out2** for the hold-up volume plus 5 mL. 
4	The retentate is recirculated for one minute using a feed pressure control of 80% of the maximum feed pressure value (to a maximum of 3 bar). 
5	The reservoir is emptied through **R-VB-Out2** (Waste). 

Flush

The system is prepared according to the procedure ***Prepare System and Reservoir*** described in *Prepare System and Reservoir, on page 109*. The transfer inlet used is either **T-VB-In2** (conditioning buffer) or **T-VB-In5** (water).

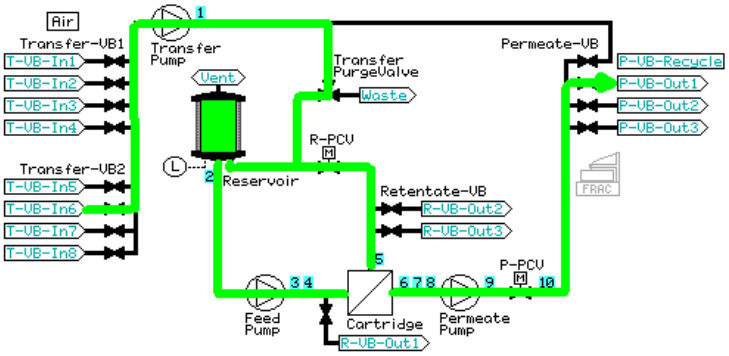
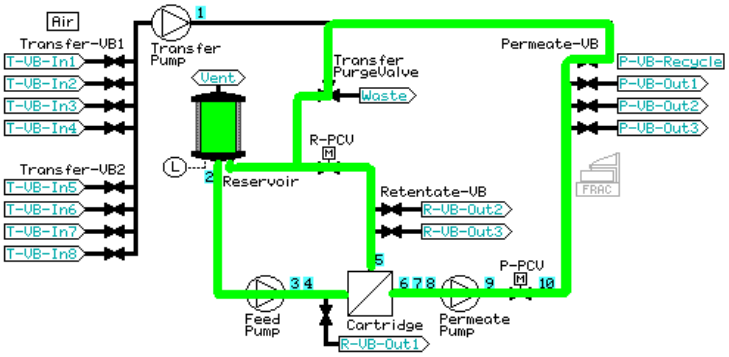
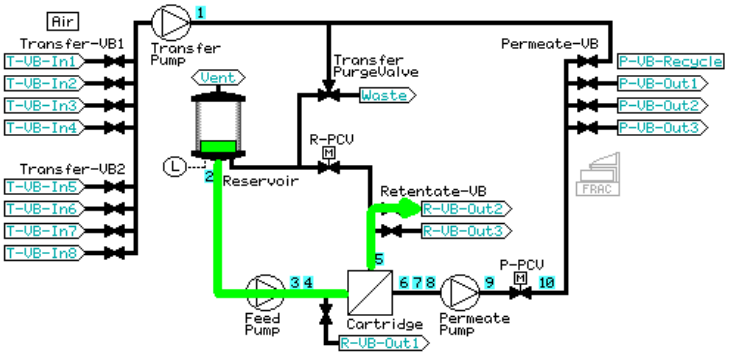
Stage	Description
1	The reservoir is filled with 100 mL solution. ***Constant Retentate Volume*** is activated. The crossflow rate is set at a feed pressure 80% of the maximum feed pressure value (to a maximum of 3 bar) and TMP regulation of 1 bar is started. The volume input in the dialog is rinsed through the filter out **P-VB-Out1** (Waste). 
2	***Constant Retentate Volume*** is disabled. 10 mL of the reservoir volume is emptied through the permeate recycle (**P-VB-Recycle**) to waste (**Transfer_Purge_Valve Waste**). 

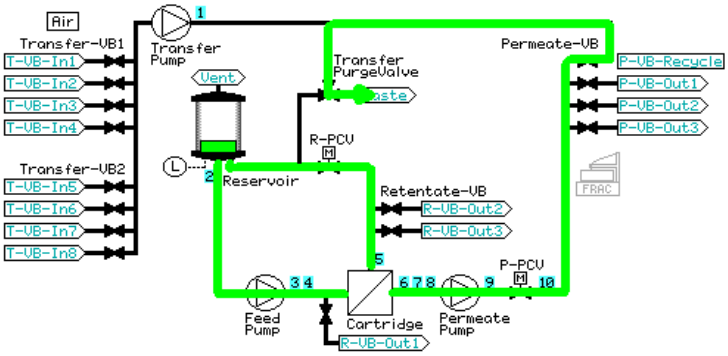
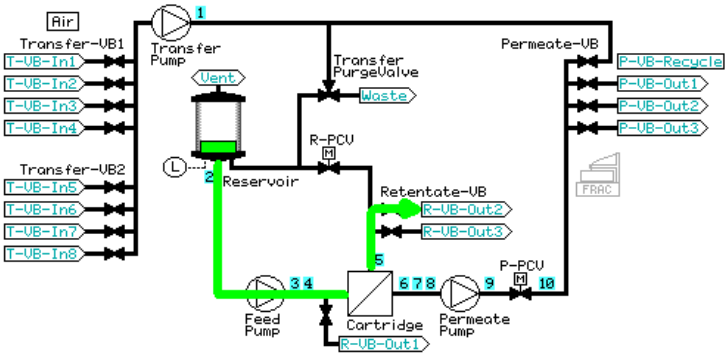
Stage	Description
3	The rest of the reservoir volume is emptied through **R-VB-Out2** and the flush is complete. 

Filter CIP

The system is prepared according to the procedure described in *Prepare System and Reservoir, on page 109*. The transfer inlet used is **T-VB-In6** or **T-VB-In7** (CIP solution), depending on whether 1 or 2 CIP steps were selected.

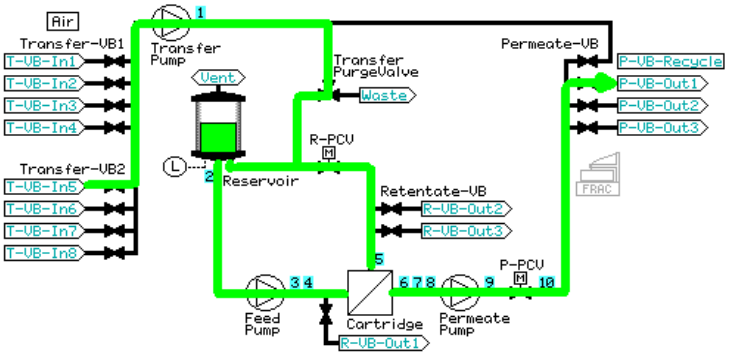
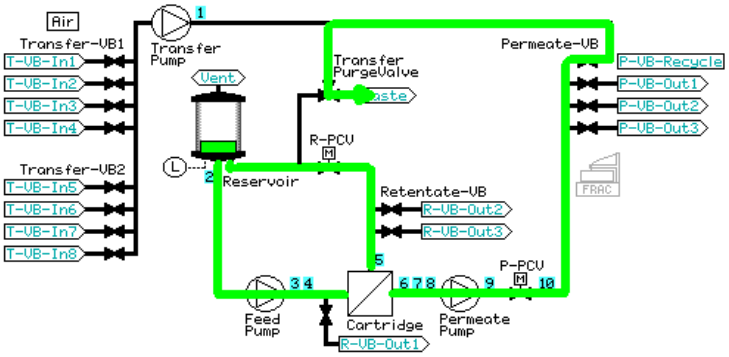
Stage	Description
1	The reservoir is filled with CIP solution to either the maximum volume (small reservoir, 350 mL) or a specified fill volume (large reservoir, minimum 200 mL (default) to 1100 mL).
2	***Constant Retentate Volume*** is then activated and the permeate valve is opened to **P-VB-Out1**. The crossflow rate is set at a feed pressure 80% of the maximum feed pressure value (to a maximum of 3 bar) and flux rate filtration control of 30 LMH (flat sheet cassette, HF microfilter) or TMP filtration control of 1 bar (HF ultrafilter) is started.

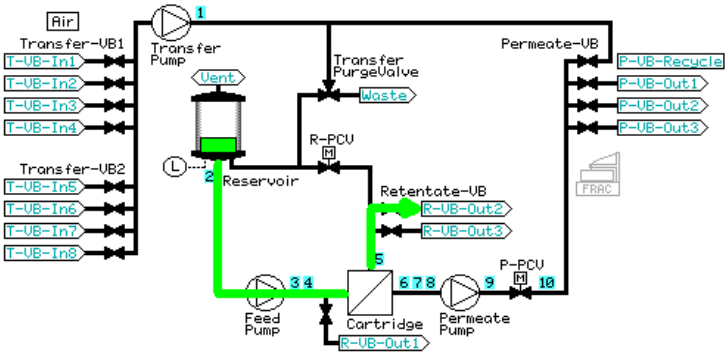
Stage	Description
3	The first 30 mL passes through the membrane out **P-VB-Out1**. 
4	After 30 mL has passed through the membrane, the permeate valve is set to **P-VB-Recycle** and the liquid is recycled back into the reservoir for the specified recirculation time. 
5	After the specified CIP recirculation time, ***Constant Retentate Volume*** is disabled and the reservoir is emptied through **R-VB-Out2**. 

Stage	Description
6	If a Water Flush between CIP has been selected, the system is prepared according to the procedure described in *Prepare system, on page 112*. The transfer inlet used is **T-VB-In5** (water).
7	The reservoir is then filled with 100 mL water and 10 mL of the reservoir volume is emptied through the permeate recycle (**P-VB-Recycle**) to waste (**Transfer_Purge_Valve Waste**). 
8	The reservoir is then emptied through **R-VB-Out2**. 
9	If a CIP 2 step has been selected, the system is prepared according to the procedure described in *Prepare system, on page 112*. The transfer inlet used is **T-VB-In7** (CIP2 solution).
10	The reservoir is filled with either 100 mL CIP 2 solution (small reservoir) or the specified fill volume (large reservoir) and the procedure described above in steps 2 to 5 is repeated.

Water flush

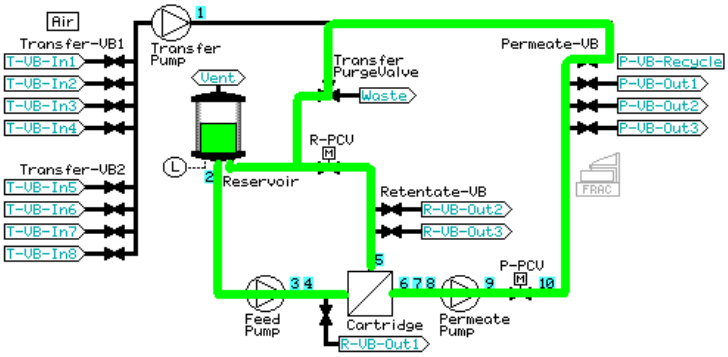
The system is prepared according to the procedure described in *Prepare System and Reservoir, on page 109*.

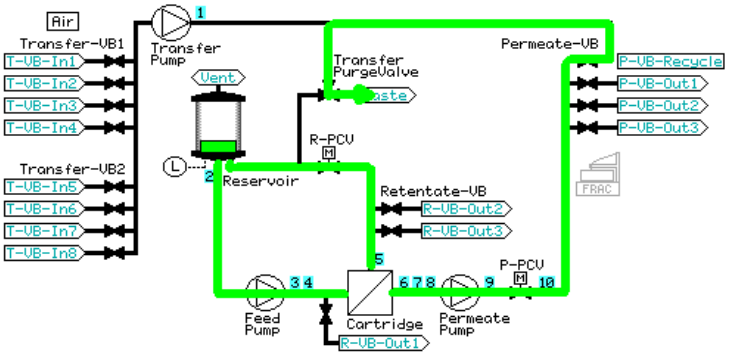
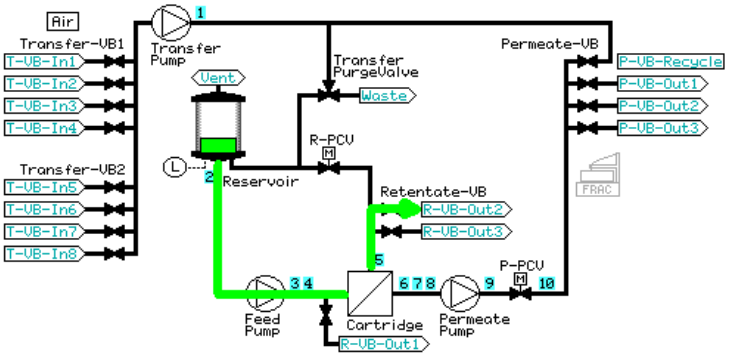
Stage	Description
1	The reservoir is filled with 100 mL water. ***Constant Retentate Volume*** is activated, and the permeate valve is opened to **P-VB-Out1**. The crossflow rate is set at a feed pressure 80% of the maximum feed pressure value (to a maximum of 3 bar) and flux rate filtration control of 30 LMH (HF microfilter) or TMP filtration control of 1 bar (HF ultrafilter, flat sheet cassette) is started. The filter is flushed with specified flush volume out **P-VB-Out2** (default volume is 2 mL per cm^2 of filter surface area.) 
2	After the specified flush through the filter, ***Constant Retentate Volume*** is disabled. If a ***Water Flux Test*** has been selected, the system skips to the test; if not, 10 mL is emptied through the filter. **P-VB** is set to ***Recycle*** and the liquid leaves the system through **Transfer_Purge_Valve** to ***Waste***. 

Stage	Description
3	The reservoir is then emptied through **R-VB-Out2**. 

Water flux test

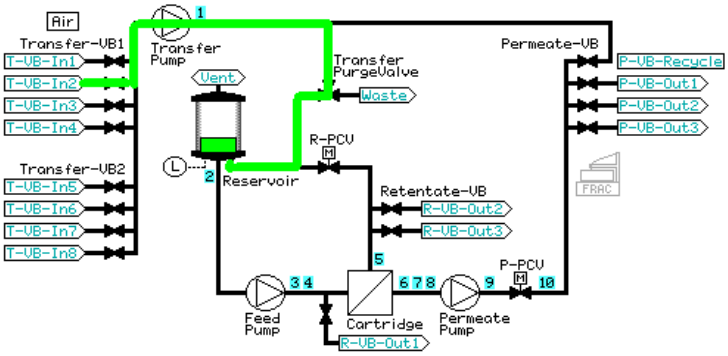
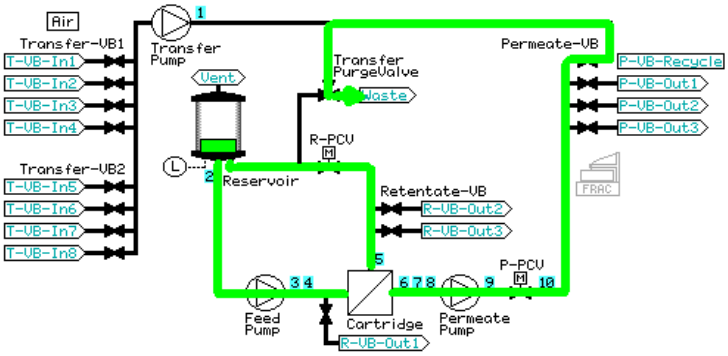
If the step prior to the water flux test is a water flush step, the reservoir remains filled and the water flux test is a continuation of the water flush step. If the water flux test is used as a stand-alone step, the system is prepared according to the procedure described in *Prepare system, on page 112*. The transfer inlet used is **T-VB-In5** (water).

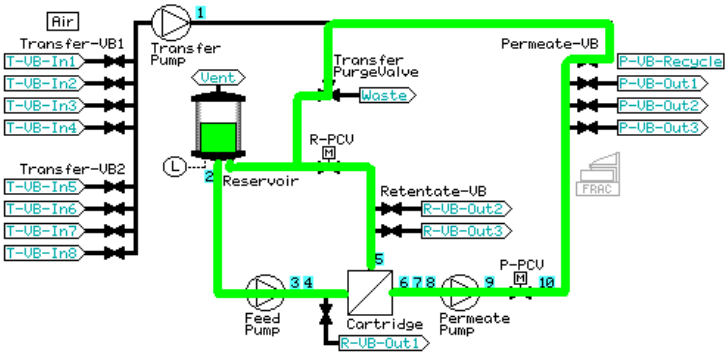
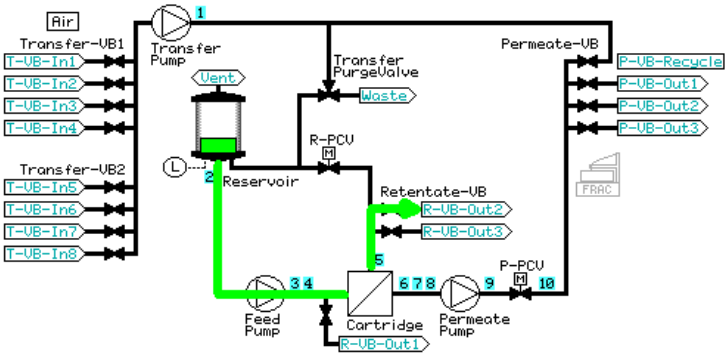
Stage	Description
1	The permeate valve block, **P-VB**, is set to ***Recycle*** and the ***Transfer_Purge_Valve*** is set to ***Reservoir***. A permeate flow is started by the specified TMP control (HF ultrafilter, flat sheet cassette; default value 1 bar) or in NFF mode (HF microfilter, default value is feed pressure, determined by the filter pore size). When a stable flux has been achieved, the normalized water flux value is measured by setting a ***Set_Eval_Mark*** with the parameter ***Normalized_Water_Flux***. 

Stage	Description
2	Before ending the method, 10 mL water is flushed through the filter. **P-VB** is set to ***Recycle*** and the liquid leaves the system through **Transfer_Purge_Valve** to ***Waste***. 
3	The reservoir is then emptied through **R-VB-Out2**. 

Storage

The system is prepared according to the procedure described in *Prepare system, on page 112*. The transfer inlet used is **T-VB-In8** (storage solution).

Stage	Description
1	The reservoir is filled with a small volume of storage solution. 
2	The permeate valve block, **P-VB**, is set to ***Recycle*** and the transfer purge valve is set to ***Waste***. The crossflow rate is set at a feed pressure 80% of the maximum feed pressure value (to a maximum of 3 bar) and flux rate filtration control of 30 LMH (HF microfilter) or TMP filtration control of 1 bar (HF ultrafilter, flat sheet cassette) is started. The specified storage rinse volume is flushed through the filter into the permeate (default value 30 mL, but this can be edited to a maximum value of 300 mL for the small reservoir or 1000 mL for the large reservoir). 

Stage	Description
3	The ***Transfer_Purge_Valve*** is then switched to ***Reservoir*** and the storage solution recycled for 5 minutes. 
4	The reservoir is then emptied through **R-VB-Out2**. 

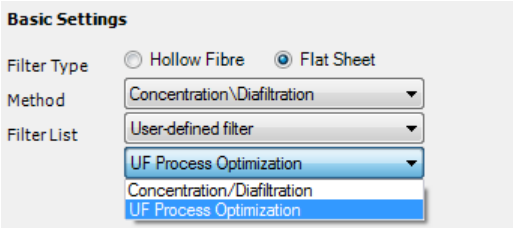
9 Process optimization in Ultrafiltration

About this chapter

This chapter provides information on using the ***UF Process Optimization*** in the ***Method Wizard***.

Introduction

When filter type ***Flat Sheet*** is selected in the ***Method Wizard Basic Settings*** dialog, a second method option (in addition to ***Concentration/Diafiltration***) is available: ***UF Process Optimization***. This choice enables the creation of a TMP excursion method that can be used to determine the optimal crossflow and TMP settings for an ultrafiltration process.



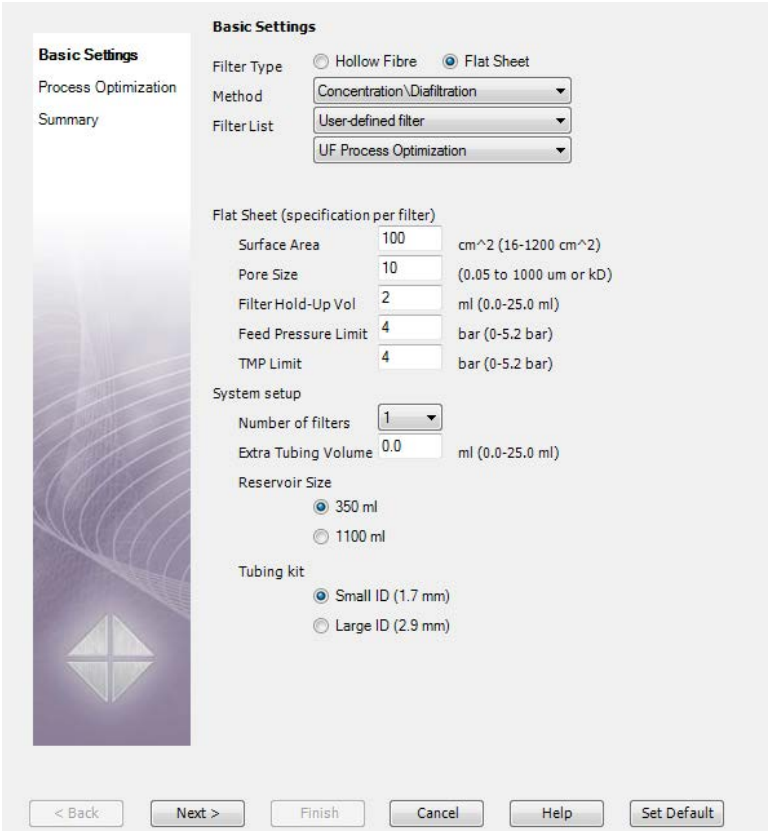
Experimental plan

As an example, a 5 mg/mL antibody is to be concentrated to 50 mg/mL using a 100 cm^2 filter. The experimental plan to determine the optimal crossflow rate and TMP for the 10 fold concentration increase includes testing 3 different feed flow rates (Q_F) with 5 TMP values at each crossflow rate. Because the optimal processing conditions can vary with concentration, the optimization is performed on both dilute and concentrated material. When testing different optimization parameters, always start with the least fouling conditions (high crossflow rate and low TMP), as the feed material will be continually recycled during the 15 combinations of crossflow rate and TMP tested.

Antibody concentration (mg/mL)		Set 1	Set 2	Set 3
5	Q_F (mL/min)	80	70	60
	TMP setpoints (bar)	0.75, 1.0, 1.25, 1.5, 1.75		
50	Q_F (mL/min)	80	70	60
	TMP setpoints (bar)	0.75, 1.0, 1.25, 1.5, 1.75		

Step	Action
1	Fill in the ***Basic Settings*** data for the flat sheet cassette: **a.** ***Surface Area*** **b.** ***Pore Size*** **c.** ***Filter Hold-Up Volume*** **d.** ***Feed Pressure Limit*** **e.** ***TMP Limit*** **f.** ***Extra Tubing Volume*** **g.** ***Reservoir Size*** **h.** ***Tubing Kit***

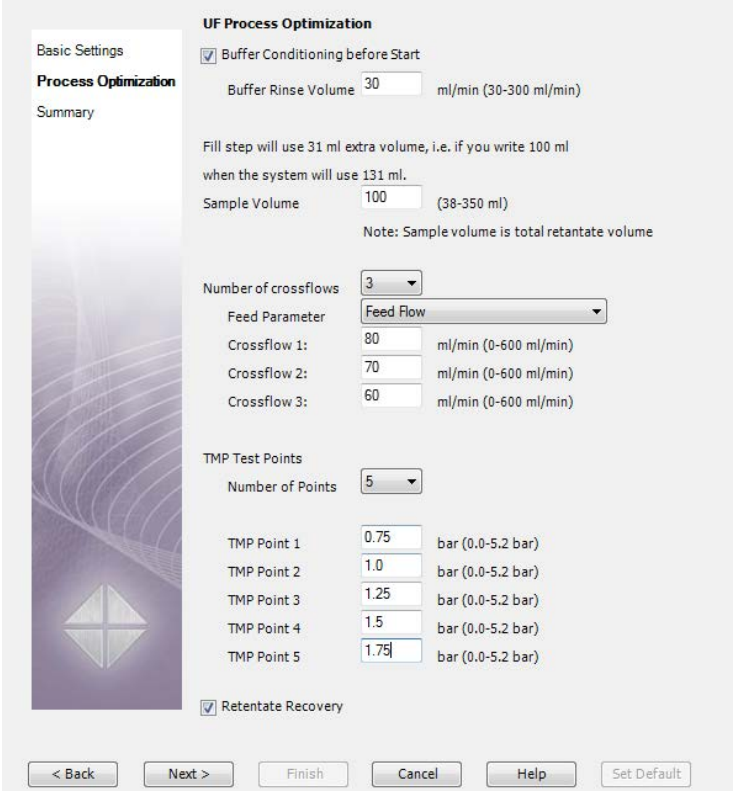
Step	Action
	Click ***Next***



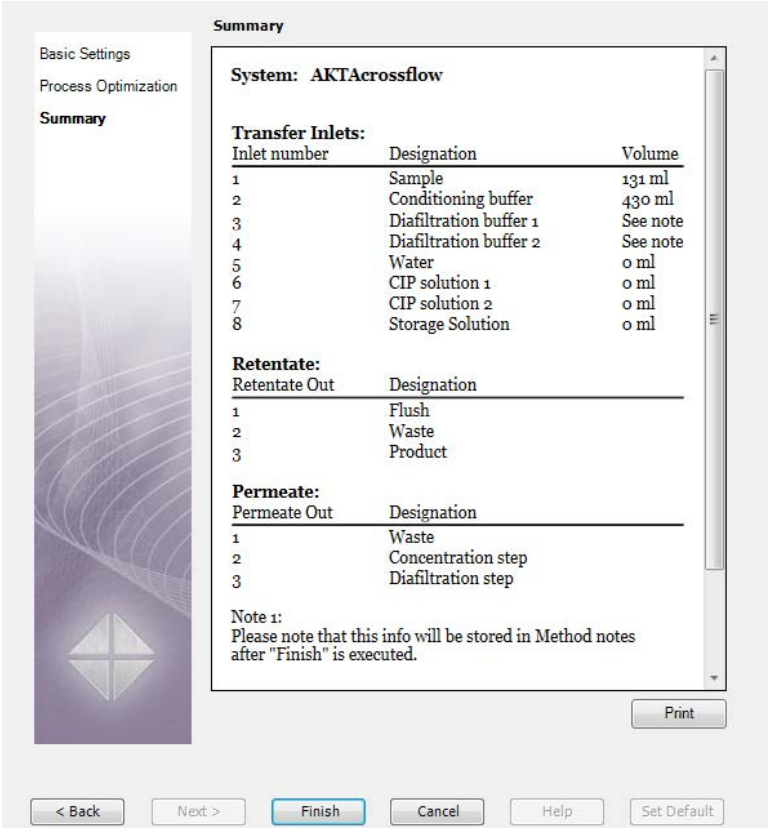
Step	Action
2	In the ***UF Process Optimization*** dialog, input the following: **a.** ***Buffer Conditioning before Start***, if desired (default rinse volume is 30 mL, but this can be edited) **b.** ***Sample Volume*** ***Note:*** *The sample volume is the total volume in the reservoir + retentate holdup volume.The system uses 31 mL extra sample volume to thoroughly flush the retentate loop, so that the sample is not diluted at the start of the process.* **c.** ***Number of crossflows*** **d.** ***Feed Parameter*** (feed flow, retentate flow, or deltaP) **e.** ***Crossflow 1***, ***2***, and ***3*** **f.** Number and value of ***TMP Test Points***

Step	Action

g. ***Retentate Recovery*** after the run (the retentate loop will be emptied according to the procedure *Recovery, no flush, on page 169*).



Step	Action
3	Click ***Next***. A summary of required solutions will be displayed. ***Note:*** *The total sample volume displayed in the Summary contains 31 mL extra sample volume for priming the recirculation loop.*



4	To save the process optimization method, select ***File*** →***Save As*** in the ***UNICORN Method Editor***.
5	Browse for a folder, enter a method name, select the system in the drop-down menu, and click ***OK***.
6	UNICORN will create a complete method, with each step contained with a sub-block of a ***User Defined*** phase. To display the text instructions of the created method, click the ***Text Instructions*** tab.

Continuing the experiment

The suggested order of experiments is:

1. Run the method with the dilute protein solution.
2. Clean the filter.
3. Concentrate the protein.
4. Run the method with the concentrated protein concentration.
5. Clean and store the filter.

Evaluating results

The ***Filtration Analysis*** option in ***Evaluation*** allows for quick plotting and analysis of the optimization run, see *Chapter 12 Evaluating ÄKTAcrossflow results using Filtration Analysis, on page 227*.

The image below shows an example result.

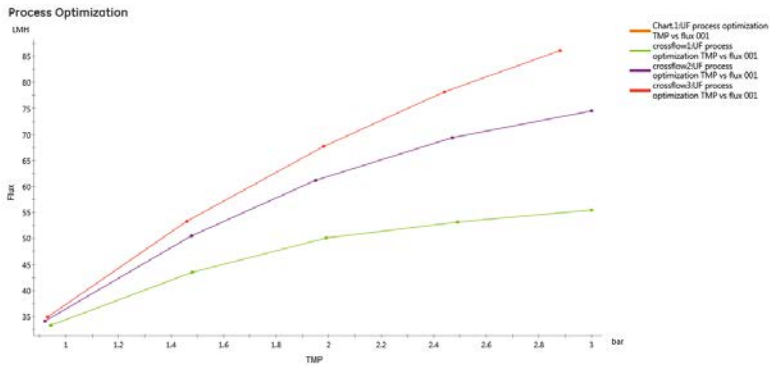


Figure 9.1: Evaluation of a TMP excursion run with a dilute glucoamylase solution

10 Running ÄKTAcrossflow methods

About this chapter

This chapter describes how to make the final preparations before starting a run, how to start the run, and what procedures to follow during the run.

In this chapter

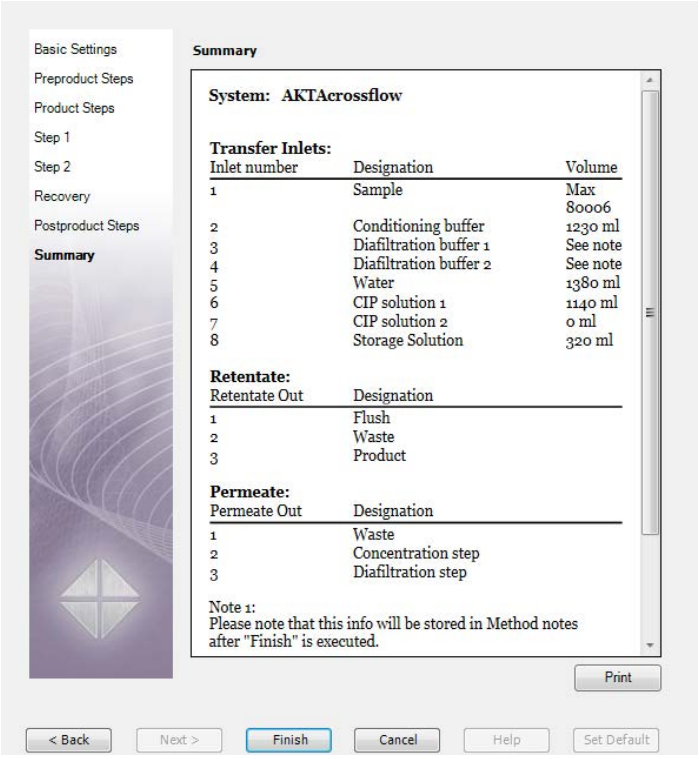
10.1 Final preparations

Introduction

This section describes the final preparations that should be done before starting a run.

The ***Summary*** page created by the ***Method Wizard*** will list the required solutions, the corresponding inlet positions, and volumes, as well as the outlet position usage.

Note: *If the air sensor terminates the sample load, the maximum sample volume will be the default 80,000 mL + 6 mL priming volume.*



Solutions

Immerse the ends of the transfer inlet tubing in the appropriate solution containers.

Check that there are sufficient solution volumes available.

Note: *Use ultra pure water when preparing solutions and buffers.*

Sample

Put the end of the sample inlet tubing into an appropriate sample container. If the air sensor will terminate the sample load, ensure that the inlet tubing reaches the bottom of the container, so that air is not prematurely detected (which will end the sample load without loading the entire sample volume).

Waste

Check that following outlet tubings are placed in waste containers:

- **Transfer Purge Valve Waste**
- **R-VB-Out2**
- **P-VB-Out1**

Check that the waste containers are not full and will accept the volume diverted to it during the run.

Filter

Check that the correct filter is properly installed, with the correct tubing for feed, retentate, and permeate. If you are blocking one of the permeate ports, we normally suggest you block the permeate port closest to the feed with a stop plug.

Make sure that the filter is clean and of acceptable quality; utilize the ***Water Flux Test*** to ensure cleaning efficiency.

Calibration

Calibrate the pH electrode and the level sensor before use. Refer to *Section 3.8 Calibrate the pH electrode, on page 53*.

WARNINGS and CAUTIONS

CIP method

When running a method using a CIP solution containing sodium hydroxide (NaOH):

WARNING

NaOH is corrosive and therefore dangerous to health. Avoid spillage and wear safety glasses, safety gloves, and protective lab coat.

CAUTION

Always ensure that the filters and system components are compatible with sodium hydroxide at the concentration, contact time, and temperature used.

General

WARNING

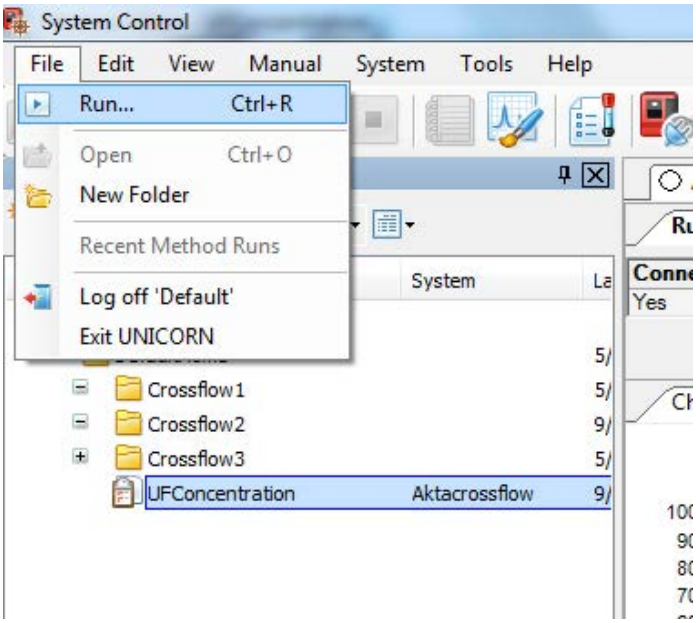
Do not operate the ÄKTAcrossflow system at pressures above the specified maximum pressure (5.2 bar).

10.2 Start a run

This section describes how to start an ÄKTAcrossflow run.

To begin the run, use the following procedure.

Step	Action
1	In the ***System Control*** module, click ***File*** →***Run***and select the method you wish to run.

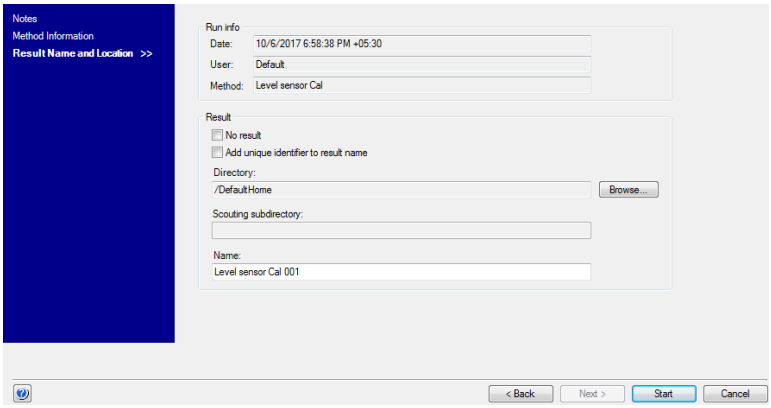


Note:

Depending on user input, a ***Start Protocol*** *may appear consisting of a number of dialog boxes. (See UNICORN Method Manual for more information).*

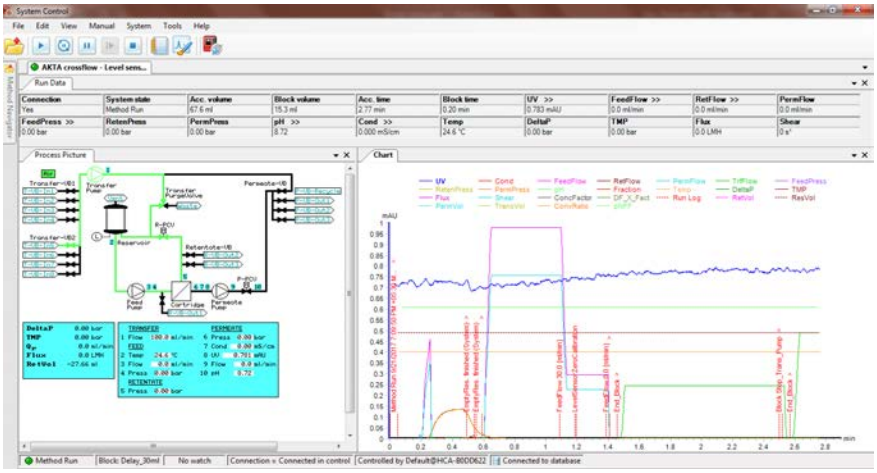
2	Click ***Next*** or ***Back*** to go through the dialog boxes.

Step	Action
3	If the ***Result Name and Location*** dialog is selected, click the ***START*** button in this screen to initiate the method run. If the ***Result Name and Location*** dialog is not selected in the ***Start Protocol***, double-click on the method name inside the folder to initiate the method run.



10.3 During the run

The method progress can be viewed in detail in UNICORN. The ***System Control*** module displays the current status of the ÄKTAcrossflow and displays up to four view panes for monitoring different aspects of the run.



To customize the view panes ***Run Data***, ***Chart***, ***Process Picture***, and ***Run Log***, drag and drop them to desired location.

For more information about customizing the view panes, see the *UNICORN System Control Manual*.

To stop the run before the end of programming, click the ***End*** button. You will be asked if you wish to save the partial run result.

Note: *If the run is in a **Hold** and is paused, you must click **Hold** to continue. Clicking **Continue** will bring the run out of **Pause** and **Hold** at the same time, see the UNICORN System Control Manual.*

10.4 Manual sampling during the run

The instruction ***ManSample*** allows sampling from the retentate in the reservoir by ensuring that concentration and diafiltration factors are correctly compensated.

To perform a manual sample, use the following procedure.

Step	Action
1	In ***System Control*** select ***Manual*** →***Execute Manual Instructions***.
2	Under ***Recirc***, select ***ManSample***.
3	Enter the planned sample volume.
4	Open the lid of the reservoir.
5	Take a sample with an appropriate pipette device.
6	Immediately, click ***Execute*** in the ***ManSample*** dialog. ***Note:*** *If **ManSample** is used when **Constant Retentate Volume** is active, the system will start to compensate the lost volume immediately. Do not delay in the execution of the **ManSample** instruction.*
7	Close the lid. ***Note:*** *To get an evaluation mark for **Filtration Analysis** in the result file, use the manual instruction **Permeate** →**Set_Eval_Mark** with the parameter **ExtData_vs_Capacity**.*

11 Post run procedures

About this chapter

This chapter describes how to sanitize the ÄKTAcrossflow after a run and how to view and print the results.

In this chapter

11.1 System sanitization

To make sure that the system is clean, for example process in run before storage, or before a new filter is used, it is recommended to sanitize the system with a suitable sanitization solution, such as 1 M NaOH. By repeating the ***System Sanitization*** method with a different solution, the method can also be used to pH neutralize the system after sanitization and to exchange the solution in the system to an appropriate storage solution, for example 20% ethanol.

CAUTION

Always remove the filter and replace with a three-way connector (18117059) when running the ***System Sanitization***. the ***System Sanitization*** method uses high flow rates and pressures that are incompatible with most crossflow filters. Replace the pH electrode with a dummy electrode and remove the reservoir float.

WARNING

The reservoir is overfilled during the sanitization. It is important to have the correct **Reservoir Cleaning Kit** tubing plumbed to waste; if this tubing is not secure, spillage will occur. NaOH is corrosive and therefore dangerous to health. Avoid spillage and wear safety glasses, safety gloves, and protective lab coat.

Note: *The stirrer should be present in the reservoir during sanitization. However, it must be replaced with a new aseptic one after the sanitization.*

Sanitization of the pump rinsing system

When performing a ***System Sanitization***, the pump rinsing system should be sanitized by replacing the 20% ethanol rinsing solution with 1 M NaOH.

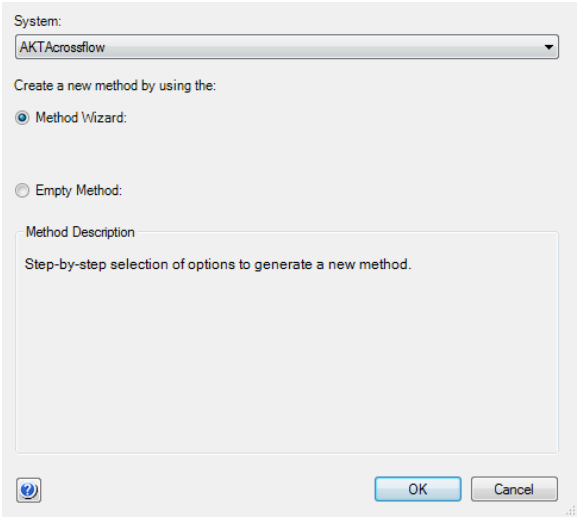
Sanitization of the reservoir float

The reservoir float must be sanitized separately. Remove it from the reservoir before the ***System Sanitization***. The float can be chemical sanitized or autoclaved.

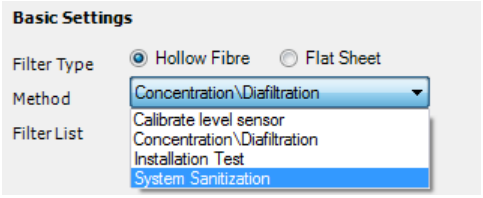
Create a *System sanitization* method

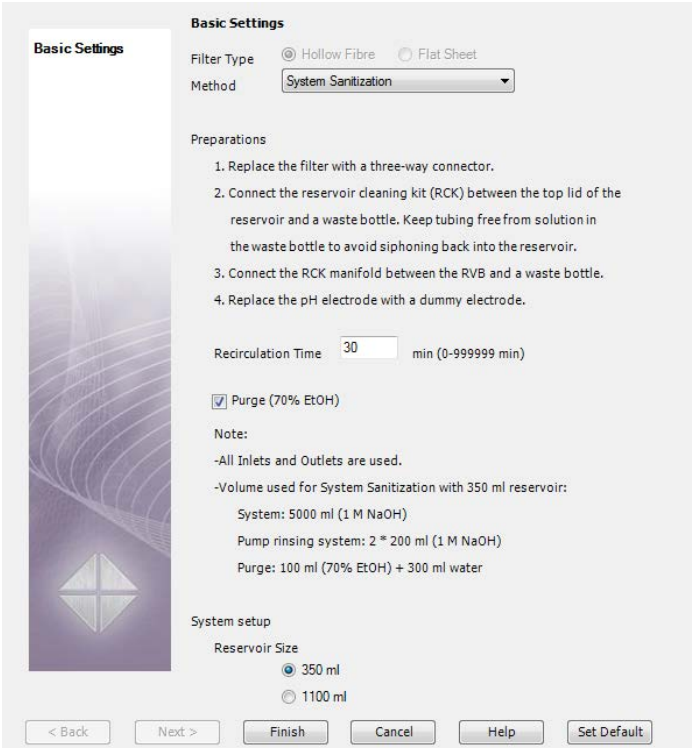
To create a ***System Sanitization*** method, use the following procedure.

Step	Action
1	In the ***Method Editor***, select the new method icon
2	Select the system.
3	Select the ***Method Wizard***.
4	Click ***OK***.

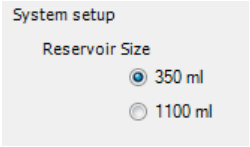


Step	Action
5	In the ***Basic Settings*** dialog, select the method ***System Sanitization***.





6	Enter a ***Recirculation Time***. A minimum recirculation time of 30 minutes is recommended.

Step	Action
7	Select the ***Reservoir Size***. 
8	An optional purge with 70% ethanol can be used to remove trapped pockets of air in the retentate valve block. If selected, you will be prompted to manually add the 70% ethanol solution to the reservoir.  ***Note:*** *The information displayed in the **Basic Settings** dialog. All inlets and outlets must be placed into the sanitization solution and all outlets will be used.* **CAUTION** 70% ethanol can require the use of explosion-proof areas and equipment.
9	Click ***Finish***.
10	Save the method.

Run the *System sanitization* method

WARNING

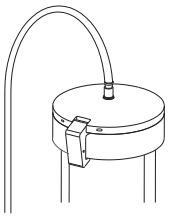
NaOH is corrosive and therefore dangerous to health. Avoid spillage and wear safety glasses, safety gloves, and protective lab coat.

CAUTION

Always make sure that the system components are compatible with the chosen sanitization solution at the concentration, contact time, and temperature used.

To run the ***System Sanitization*** method, use the following procedure.

Step	Action
1	Prepare a sanitization solution which is compatible with the ÄKTAcrossflow system, for example 1 M NaOH. If using the small (350 mL) reservoir, 5000 mL is required; if using the large (1100 mL) reservoir, 7000 mL is required.
2	Fill the pump piston rinsing bottles with 1 M NaOH (2 × 200 mL).
3	Replace the filter with a three-way connector.
4	Replace the pH electrode with a dummy electrode.
5	Remove the float from the reservoir.
6	If an air filter is connected to the reservoir, remove the filter. Connect the ***Reservoir Cleaning Kit*** (11003386) tubing to the closed lid of the reservoir.



Note:

To avoid siphoning waste liquid back into the reservoir, keep the reservoir tubing above the liquid of waste solution in the waste bottle.

7	Place all inlet tubing into the prepared sanitization solution.
8	Place all outlet tubing into a waste container.

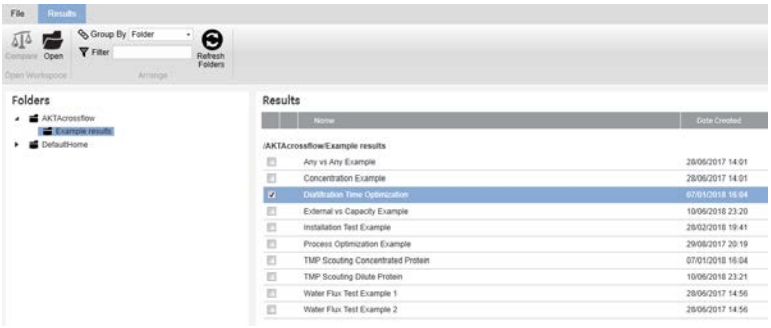
Step	Action
9	Run the method. ***Note:*** *at the end of the **System Sanitization** method, the system will remain in sanitization solution which must be rinsed out for further use or for storage. Do not store the ÄKTAcrossflow in NaOH.*
10	After the ***System Sanitization*** method, empty the system rinsing bottles and fill them with 20% ethanol.
11	Repeat the method using ultra pure water or buffer instead of sanitization solution.
12	After the run with ultra pure water or buffer, empty the system rinsing bottles and fill them with 20% ethanol.
13	Replace the stirrer with a new aseptic one.

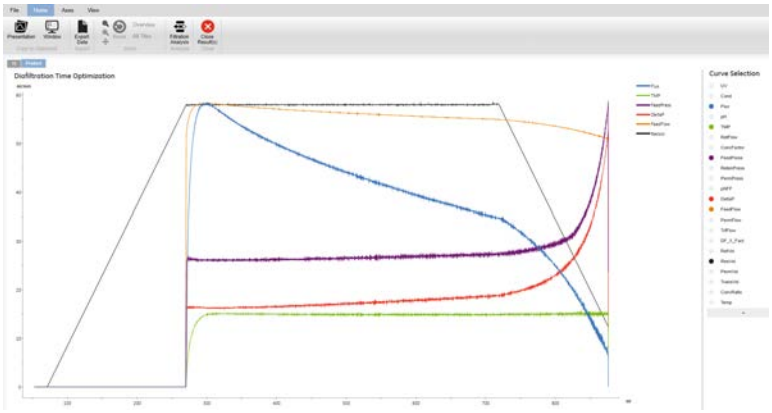
11.2 Viewing and printing the result

This section describes the basics of how to view and print the result in the ***Evaluation*** module.

View the result

To view the result, use the following procedure.

Step	Action
1	Double click on the ***Evaluation*** icon to open the UNICORN ***Evaluation*** module. 
2	Locate the result file in the ***Results*** folder. 

Step	Action
3	Double-click the file. *Result:* file opens in a chart window in the ***Evaluation*** module. 
4	Charts can be included or excluded for analysis from the chart selection pane. 

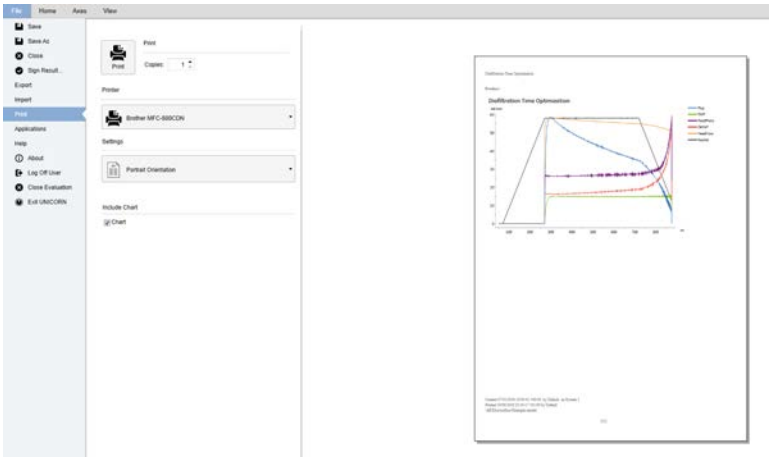
Refer to the *UNICORN Evaluation Manual* for more information.

Print the result

To print the chart, use the following procedure.

Step	Action
1	Select the chart you want to print in the chart selection pane.

Step	Action
2	Select the ***File*** →***Print***command.



3	Select the number of copies, printer, orientation, and check ***Include Chart***.
4	Click on ***Print***.

Refer to the *UNICORN Evaluation Manual* for more information.

Evaluation Classic

For more complex actions, such as report creation, procedures, and setting vertical markers over a reference area (for example, to average flux rates across a product run), ***Evaluation Classic*** is available under a separate UNICORN license. Refer to the *UNICORN Evaluation Manual* for more information.

12 Evaluating ÄKTAcrossflow results using ***Filtration Analysis***

The UNICORN ***Evaluation*** module contains a special analysis tool for filtration runs, called ***Filtration Analysis***. Five different operations are available for rapid analysis of runs performed on the ÄKTAcrossflow system:

- ***Process Optimization***
- ***Diafiltration Time Optimization***
- ***Normalized Water Flux***
- ***Capacity Plots***
- ***Any vs Any***

About this chapter

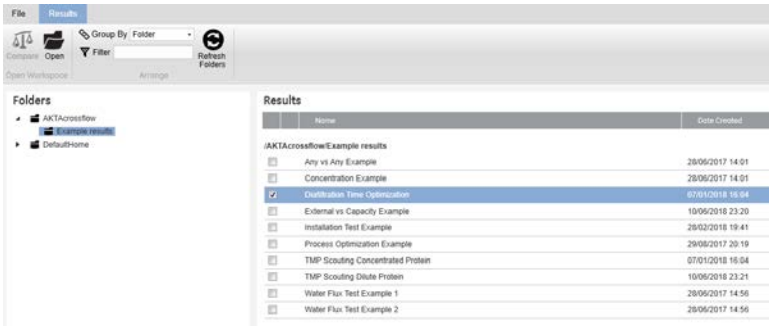
This chapter describes how to analyze results from the run using the UNICORN ***Evaluation*** module.

In this chapter

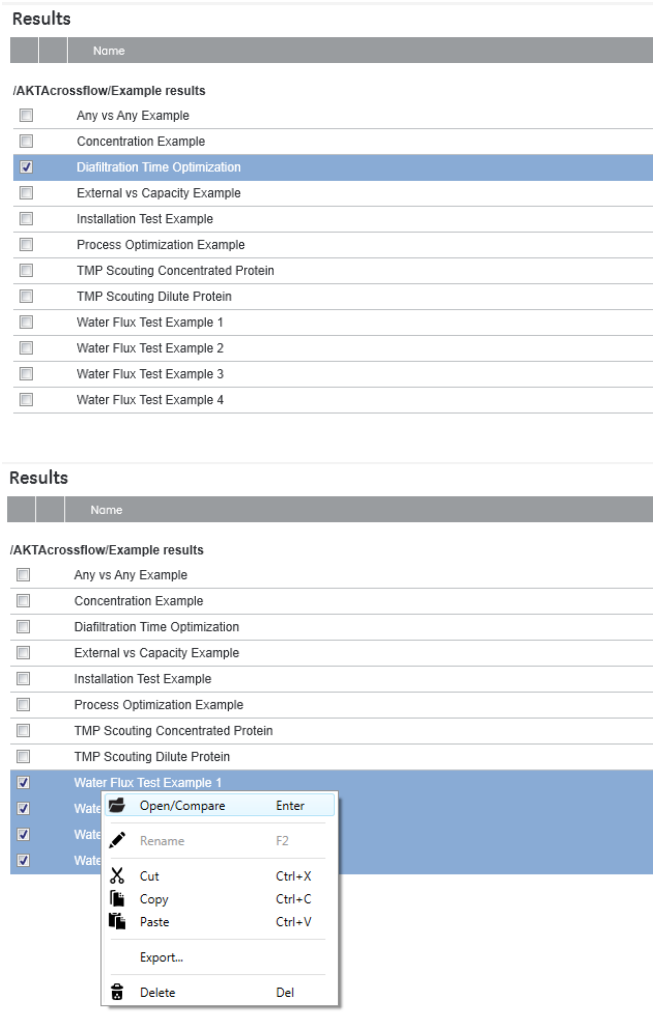
12.1 Open a result file in the ***Evaluation*** module

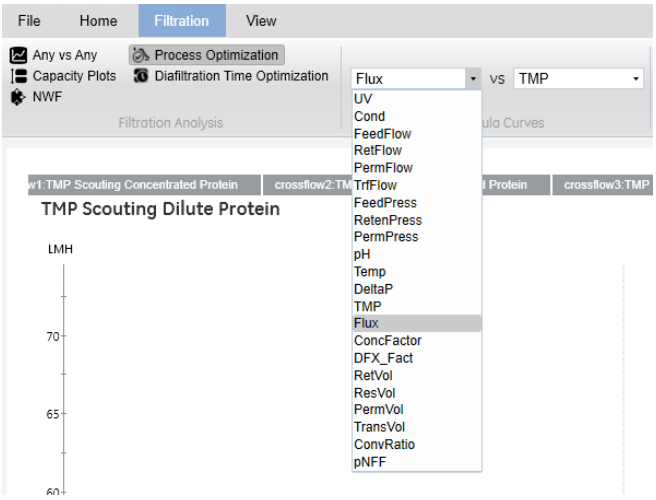
To start the ***Filtration Analysis***, first open a result file in ***Evaluation***. To do this, use the following procedure:

Step	Action
1	In the UNICORN ***Evaluation*** module, select the result to be analyzed in the result browser.

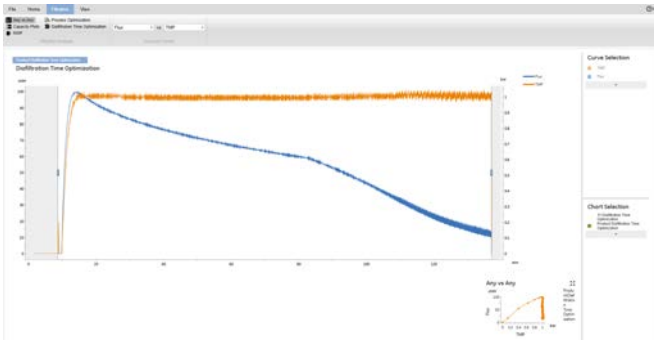


Step	Action
2	Double-click on one result file or right-click on multiple result files to compare the selected results.



Step	Action
3	Once the result file(s) is open, click on the ***Filtration Analysis*** button to start the analysis.  ***Note:*** *If the result file is being analyzed for the first time, UNICORN will request first to close the opened result to enter the* ***Filtration Analysis*** *module.*
4	Close the opened result to enter the ***Filtration Analysis*** module. The ***Any Vs Any*** algorithm is be applied to the selected results by default. ***Note:*** *For each operation, specific curves for that operation are selected by default, but these are user editable, by clicking on* ***Formula Curves****.* 

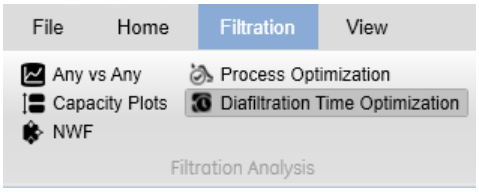
Step	Action
5	Select/deselect the curves and charts to be displayed in ***Curve Selection*** and ***Chart Selection*** on the right hand side.



Note:

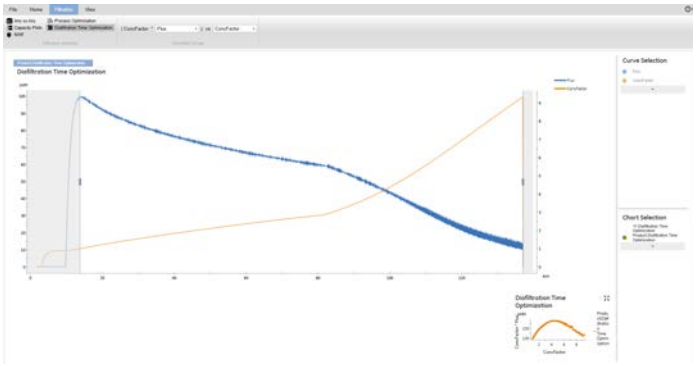
ÄKTAcrossflow methods created by the ***Method Wizard*** *contain multiple charts to split the results between preproduct, product, and postproduct steps. Additionally, each method begins with a chart 11, which is the data generated at the beginning of a method wizard run in which the reservoir is emptied to start the run. This can be de-selected as standard.*

Step	Action
6	To apply a particular type of analysis, click on the analysis name in the ***Filtration Analysis*** selection box.



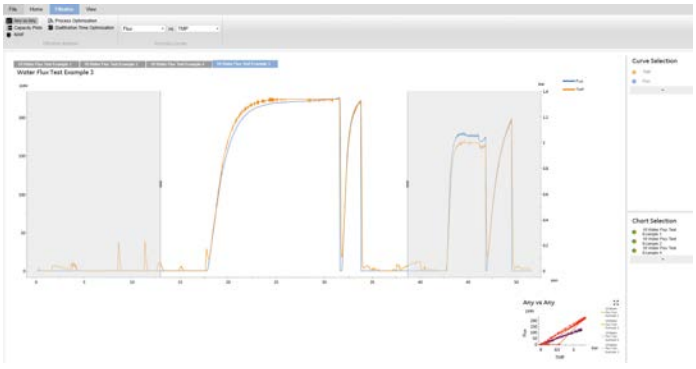
Result:

The chart now shows the selected analysis.

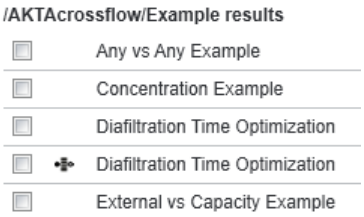


Note:

If multiple files have been opened, the results appear as separate charts which can be selected or removed by clicking on ***Chart Selection*** *on the right hand side.*



Step	Action
7	Analyses can be saved, and are denoted in the ***Results*** list with a ***Filtration Analysis*** symbol.



12.2 Analysis operations

In this section

12.2.1 Process optimization

Process optimization is used to analyze a special type of process characterization where a series of setpoints are tested. The most common experiments are excursions of TMP at different feed conditions, such as crossflow rates or protein concentration. In this operation, a new plot is made from user-defined points along original data curves (e.g., permeate flux vs. TMP). Process optimization also allows the user to overlay multiple plots (e.g., flux vs. TMP at different crossflow rates or protein concentrations). This capability can be used for any process parameter, but is most often used to determine the optimal crossflow rate and TMP for a product concentration/diafiltration step.

Generally, permeate flux increases with increasing TMP. However, as a concentration polarization layer is formed at the membrane surface, the flux vs. TMP curve flattens. Increasing the TMP beyond this flattening often leads to a flat line or decreasing curve, where increasing the TMP does not increase the permeate flux, due to formation of a gel layer on the membrane surface and subsequent control of the gel layer over the filtration process.

With this operation, data from up to 3 crossflow rates per result (with multiple TMP values) can be overlaid.

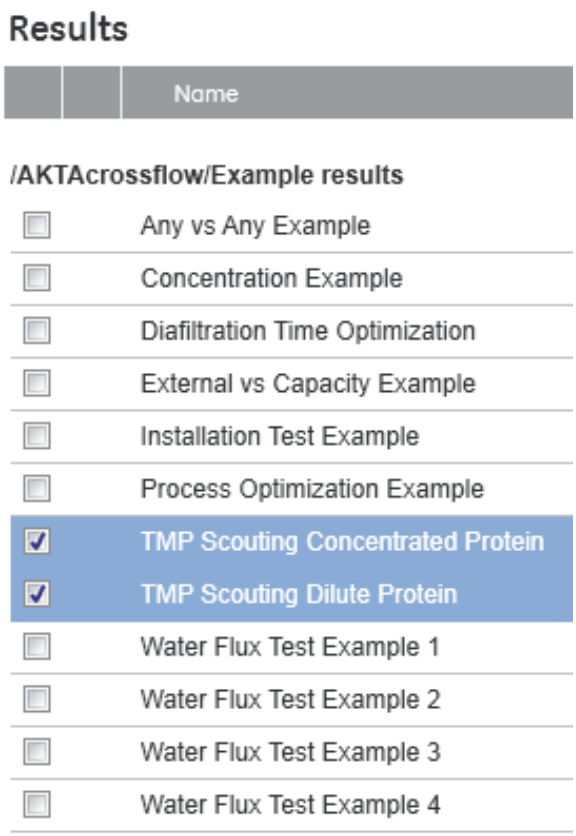
Tip: For a comparison of more than 3 crossflow rates, split the optimization method into multiple runs. The multiple results can then be opened and compared, allowing filtration analysis to be performed on all crossflow rates in one operation.

To start the ***Process Optimization***, use the following steps:

Step	Action
1	*Either:* • Double-click on a single ***Flat Sheet UF Process Optimization*** result file or • select multiple ***Flat Sheet UF Process Optimization*** result files, right click, and ***Open/Compare***.

Note:

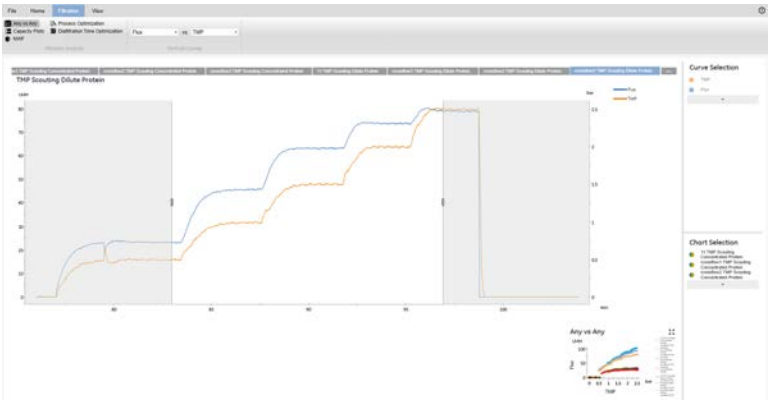
In this example, two results generated from flat sheet cassette ***UF Process Optimization*** *methods are opened, one from dilute protein tested at 5 different TMPs each for 3 crossflow rates, and the second from concentrated protein tested at the same 5 different TMPs each, for the same 3 crossflow rates.*



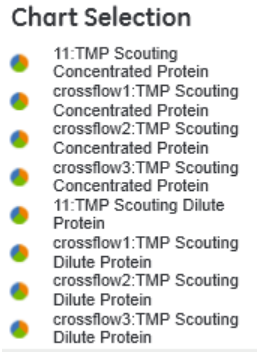
Step	Action
2	Click on ***Filtration Analysis*** and if prompted, close without saving.

Result:

The result files are opened under the default ***Any vs Any*** operation.



3	Deselect the two ***11*** charts by clicking on the multi-colored icon next to their names.



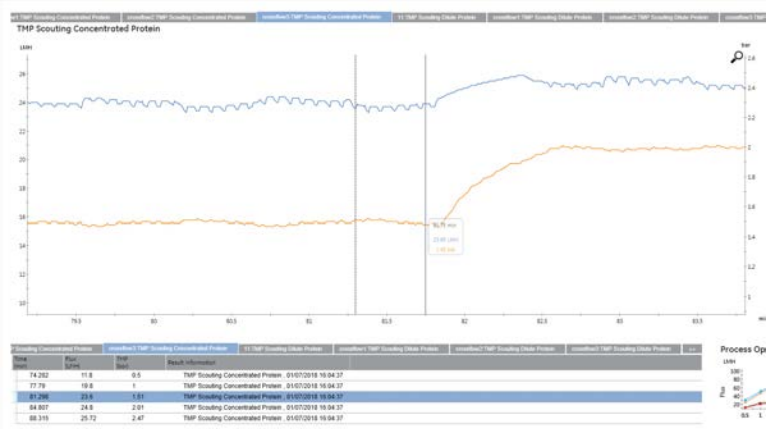
4	Select the ***Process Optimization*** operation.

Step	Action
5	In the ***UF Process Optimization*** method, as each TMP stabilized, the method inserted an evaluation mark with the instruction ***Set_Eval_Mark*** for ***ProcessOptimisation*** . In the section at the bottom of the page, select each chart to see where the permeate flux evaluation marks were made in the optimization run.
6	For each chart, click on a data point in the table to create a vertical marker in the chart.

Note:

The data point can be changed if, based on visual determination of the flux curve, another point on the curve is more suitable. Simply bring the mouse cursor into the chart; a vertical marker appears. Right click to add another data point to the table. Click on any table entry that you would like to exclude. Scroll through all charts to make any desired changes to the data points in the table.

Step	Action
7	For better visual inspection, click and drag on the chart to zoom into the selected area.

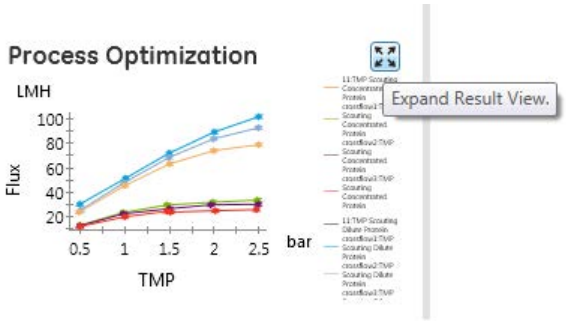


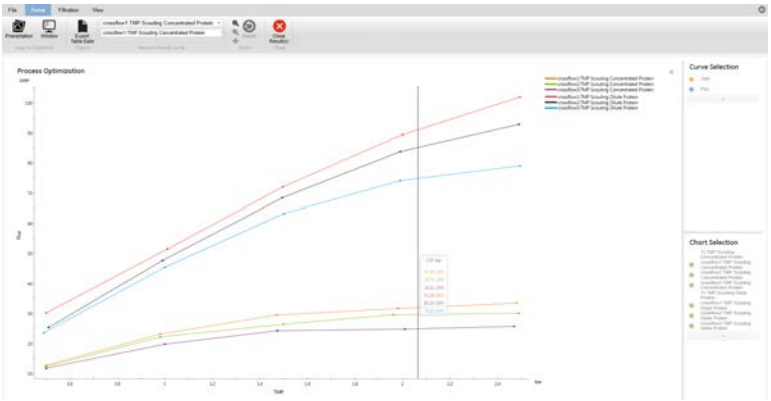
Step	Action

Note:

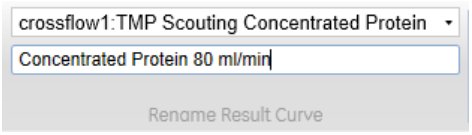
A magnifying glass icon will appear in the upper right corner. Click the icon to zoom out to the full chart again.

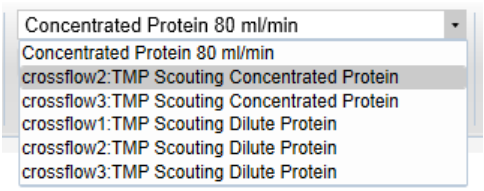
8 Once you are satisfied with the data point selection, click on the ***Expand Result View*** in the bottom right hand corner.

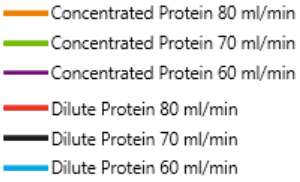




Step	Action
9	Visually determine the most suitable crossflow rate and TMP value for a run by observing the change in the permeate flux curve with increasing TMP values.







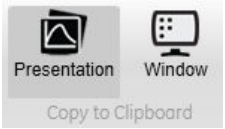
Note:

The most appropriate combination of crossflow rate and TMP can be seen where the flux is still increasing with increasing TMP, before the flux curve starts to flatten. Additionally, under ***View*** *→****Data Points****, a vertical marker appears by moving the mouse cursor into the pane, showing the permeate flux rates (color-coded to the individual charts) at a certain TMP value.*

10 To present your data, utilize ***Copy to Clipboard***. There are two options:

a. ***Presentation***, which copies the chart to the clipboard in a presentation size format.

b. ***Window***, which copies the chart to the clipboard in screen size (same as Ctrl+C).

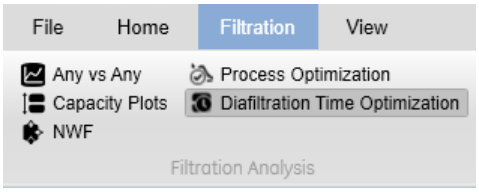


12.2.2 Diafiltration time optimization

For a given ultrafiltration process, the operation ***Diafiltration Time Optimization*** allows the user to identify the factor of volume concentration where the least time is required to complete the diafiltration. This is a function of the increase of concentration vs the subsequent decrease in permeate flux.

Diafiltration Time Optimization creates a plot of the diafiltration time optimization parameter (concentration factor × flux) vs concentration factor. The concentration factor that corresponds to the highest value of the DF time optimization parameter (y) along the plot is the optimal concentration factor to perform a diafiltration (for the conditions tested).

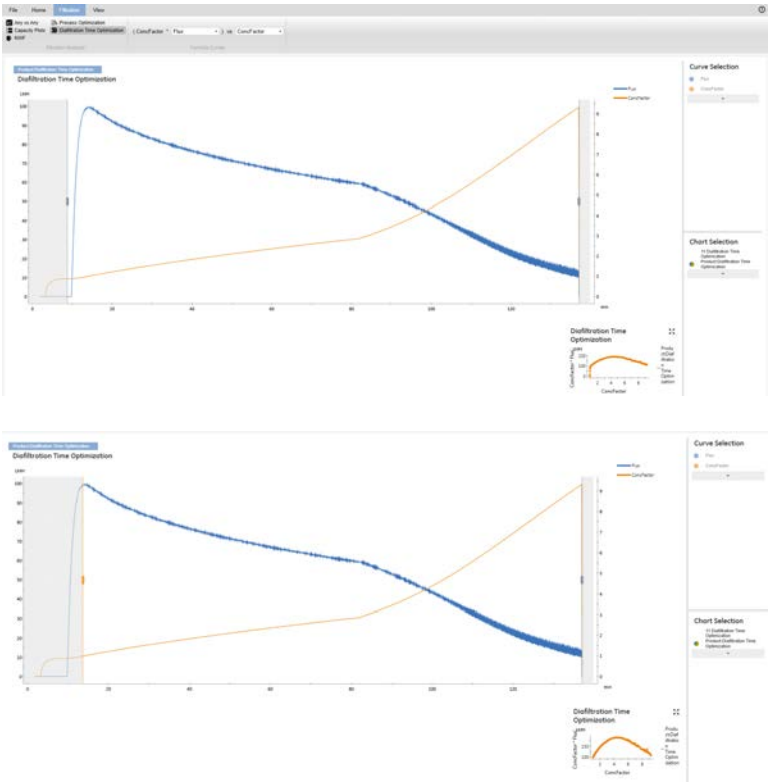
This filtration analysis is performed on a result file from a concentration process which was run to the desired maximum concentration factor. To start the ***Diafiltration Time Optimization*** analysis, use the following procedure:

Step	Action
1	Double-click on a result file in which a concentration to maximum desired concentration factor has been performed.
2	Click on ***Filtration Analysis*** and if requested, exit without saving. 
3	Deselect any chart that does not contain the concentration step.
4	Click on the ***Diafiltration Time Optimization*** operation. 
5	Deselect the chart ***11*** under ***Chart Selection*** by clicking on the multi-colored icon next to their names

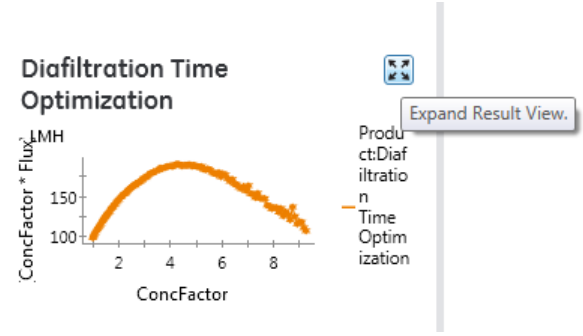
Step	Action
6	Set the left and right boundary limits for the data. To see the effect that the increasing concentration has on the permeate flux rate, use the left mouse button to drag the boundary marker to the high point of the permeate flux on the left.

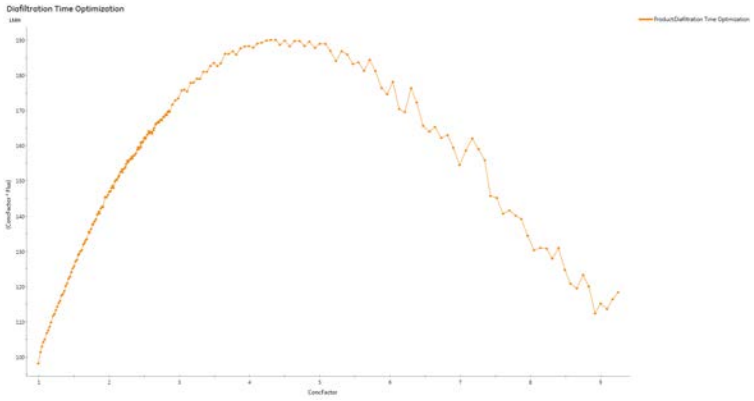
Note:

The plotting region between the left and right markers is defined by ***Start_Eval_Window*** *and* ***Stop_Eval_Window*** *instructions in a concentration step of a product method.*



Step	Action
7	Click on ***Expand Result View*** to observe the plotted graph of (concentration factor × flux) vs concentration factor.





8	Determine the concentration factor in which the diafiltration should be performed by visual assessment. Make note of the concentration factor that corresponds to the highest point on the y axis.

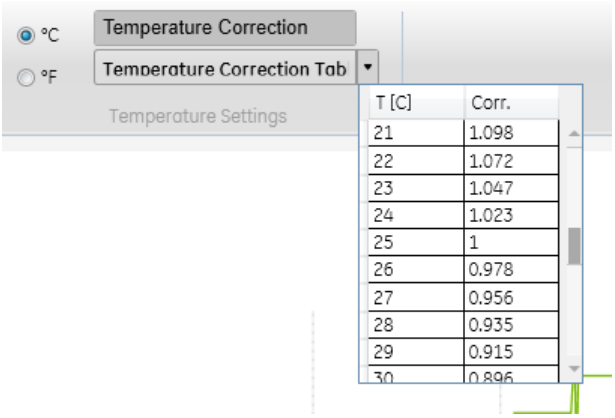
12.2.3 *Normalized Water Flux* (NWF)

The membrane permeability can be tested using the ***Normalized Water Flux*** operation (also called the normalized water permeability or NWP, or clean water flux). This test is used to make sure that the cleaning is effective and to determine the lifetime of a filter. The ***Normalized Water Flux*** is calculated using the following formula:

Normalized water flux [$Lm^{-2}h^{-1}bar^{-1}$] = (permeate flux × temperature correction factor)/TMP

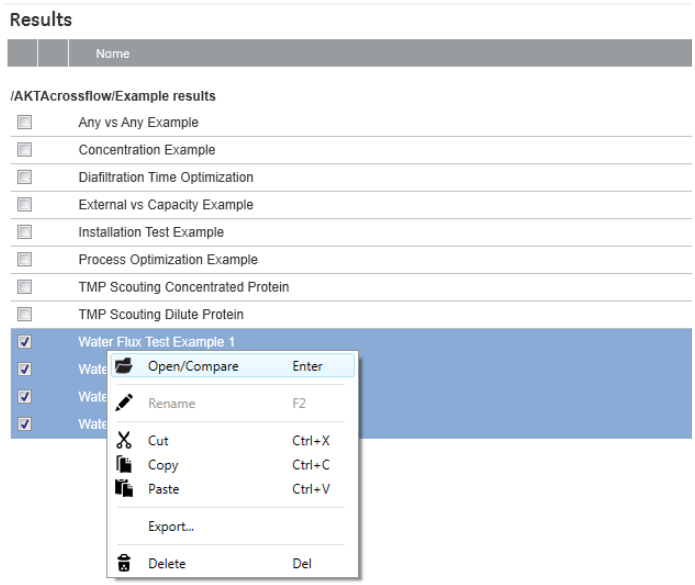
The ***Normalized water flux*** operation enables the user to automatically calculate the normalized water flux from a result file and to plot results from multiple filter cycles on a single plot.

An industry standard temperature correction chart for crossflow filtration processes is used. Temperature can be displayed in either Celsius or Fahrenheit.



To test the membrane permeability with the ***Normalized Water Flux*** operation, use the following procedure:

Step	Action
1	*Either:* **a.** double-click on a single result file containing a ***Water Flux Test*** step,*or* **b.** select multiple result files, right click, and ***Open/Compare***.

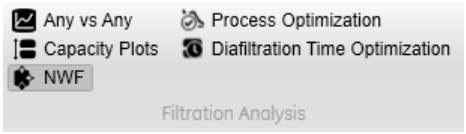


Note:

In this example, four result files that contain ***Water Flux Test*** *steps are opened.*

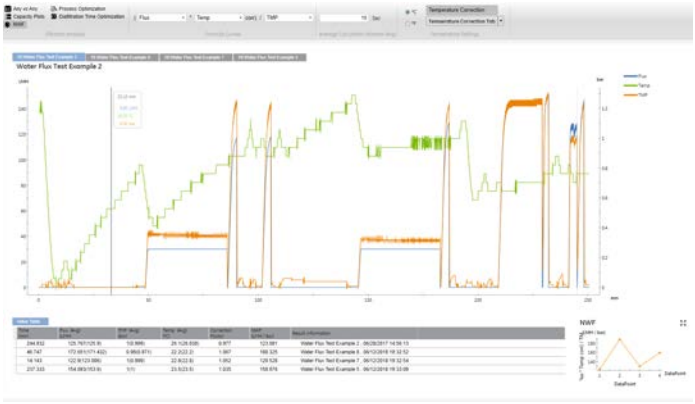
2	Click on ***Filtration Analysis*** and if requested, exit without saving.

Step	Action
3	Select the ***NWF*** operation.

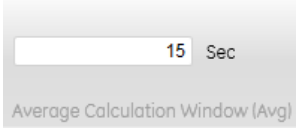


Note:

The resulting graph contains a ***Value Table*** *in which the normalized water flux values are listed, in order of date and time stamp.*



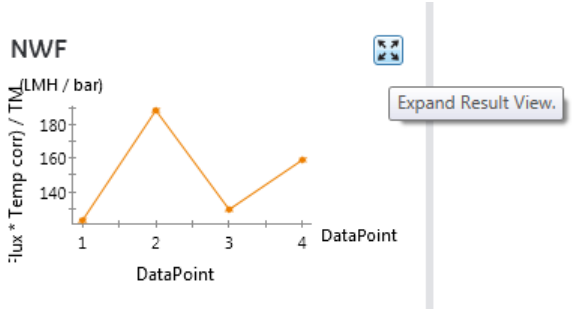
4	If required, enter a new value in the ***Average Calculation Window (Avg)***.



Note:

The default value for the calculation window is 15 seconds before the marker position. The values are expressed as the exact flux at the marker point, with averages in parentheses based on the ***Average Calculation Window (Avg)****. The values in the* ***Value Table*** *will automatically update, including the* ***Correction Factor****, which is based on the averaged values.*

Step	Action
5	Click on ***Expand Result View*** to observe the normalized water flux values plotted over time, in order of result date and time stamp.



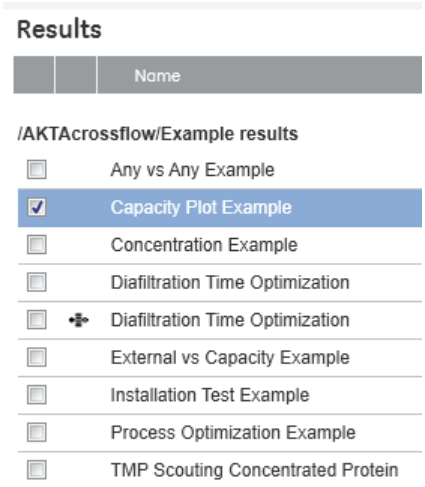
12.2.4 Capacity plots

Capacity Plots allow the user to plot any process parameter vs the accumulating permeate volume normalized to the surface area. Capacity is defined as liters of permeate volume per m^2 surface area.

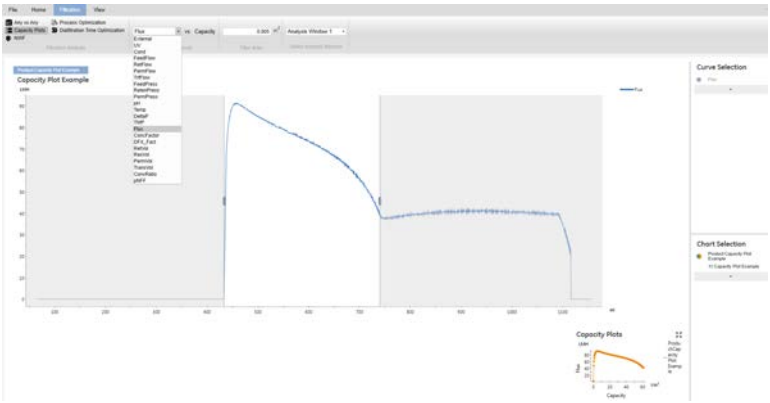
The capacity plot operation also accepts input of a system-external result from sampling during a run (e.g., activity assay results or protein concentration determination). This enables plotting of the external result vs capacity.

To start the process, use the following steps.

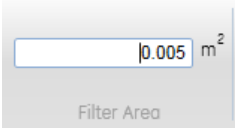
Step	Action
1	Double-click on a result file containing either a concentration or a diafiltration product step. ***Note:*** *Although most capacity plots are used for product steps, this operation can be used on any result file to plot any process parameter vs capacity. The example below will show the use of the capacity plot operation on a product step.*
2	Click on ***Filtration Analysis*** and if requested, exit without saving.
3	Deselect any chart which does not contain a product step.



Step	Action
4	Select the desired curve to plot against capacity. For example, in a concentration step, permeate flux can be plotted as a function of filter capacity. Adjust the left and right boundary limits to include the desired data.

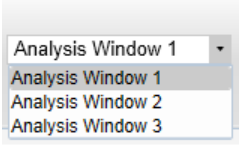


5	The filter area is imported from the result and is used to calculate capacity. It can, however, be edited, if desired.

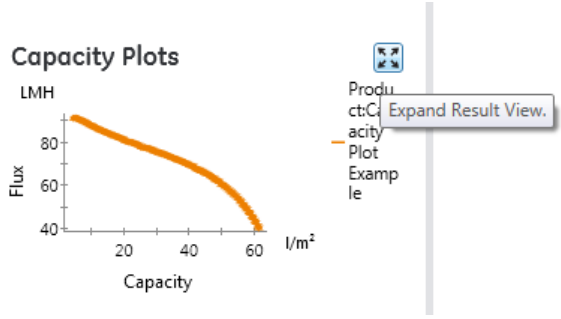


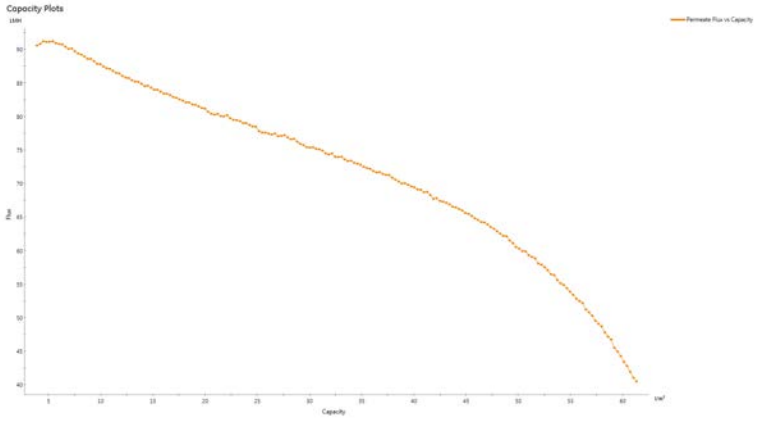
Note:

A result file can contain more than one analysis window. When a product method is created by the ***Method Wizard****, every product step is defined by* ***Start_Eval_Window*** *and* ***Stop_Eval_Window*** *instructions. The possible analysis windows in this result represent a 3-product step process: concentration, diafiltration, and concentration.*



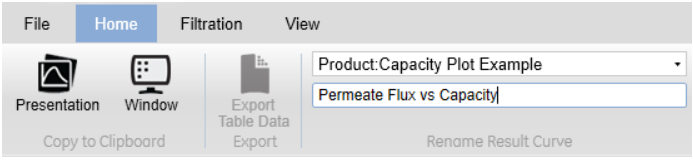
Step	Action
6	Click on ***Expand Result View*** to observe the chosen curve (in this example, permeate flux) vs capacity.



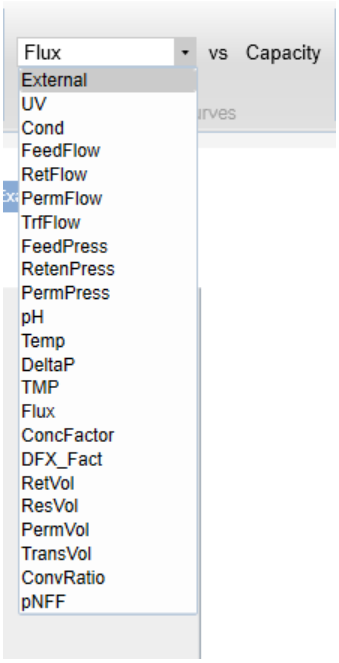


Note:

Chart names can also be changed under ***Home*** *→****Rename Result Curve*** *by selecting the result name in the upper box and editing the name in the lower box.*



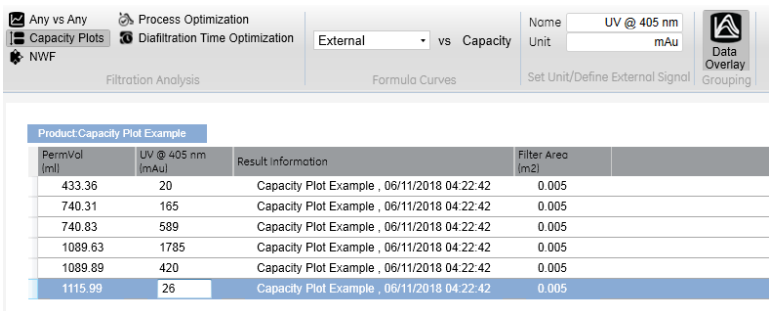
Step	Action
7	To plot ***External Data vs Capacity***, select ***External*** in the drop-down menu.



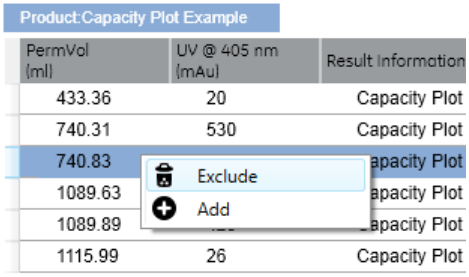
Result:

A table will open, into which the external data measured can be entered offline (e.g., activity assay results or protein concentration).

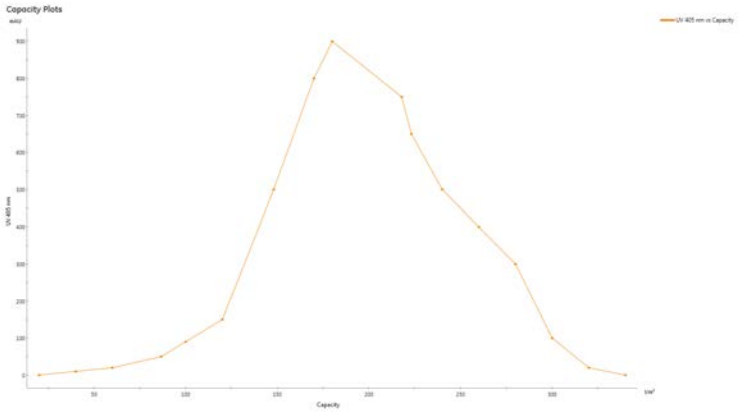
8	Enter the permeate volume at which the offline measurement was made and the value of the offline measurement by double-clicking in the pane under the appropriate columns (***PermVol*** and ***External***).



Step	Action
9	Enter the name of the external signal and the unit in the ***Set Unit/Define External Signal*** box.
10	To exclude a data point, select the data point, right click, and click ***Exclude***.



11	To add additional data points during the run, in ***System control*** click ***Manual*** →***Execute*** →***Manual Instructions*** →***Set Mark***.
12	Click on ***Expand Result View*** in the bottom right hand corner to view the plot.



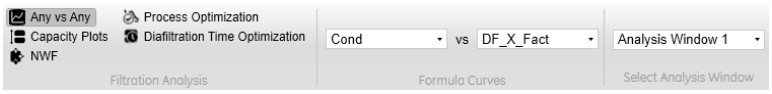
12.2.5 *Any vs Any*

The ***Any vs Any*** operation provides the capability to plot any process parameter captures as a curve in a given result file on either the x axis against any other process parameter captured as a curve on the y axis.

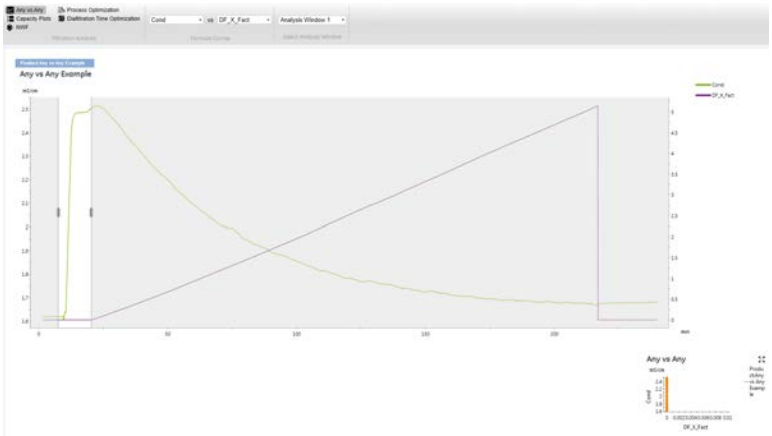
To perform this operation, use the following steps:

Step	Action
1	Double-click on a result file.
2	Click on ***Filtration Analysis*** and if requested, exit without saving.  ***Note:*** ***Any vs Any*** *is displayed by default.*
3	Deselect any chart which does not contain information of interest.
4	To choose the curves to plot on the x and y axes, select the drop-down menus in the ***Formula Curves*** box. ***Note:*** *If using the* ***Any vs Any*** *operation with a result generated from a method created by the* ***Method Wizard*** *that contains product steps (concentration and diafiltration), each step is defined by* ***Start_Eval_Window*** *and* ***Stop_Eval_Window*** *instructions.*

Step	Action
5	If analyzing a product run with multiple analysis windows, choose the appropriate window in the drop-down menu in the ***Select Analysis Window*** box.

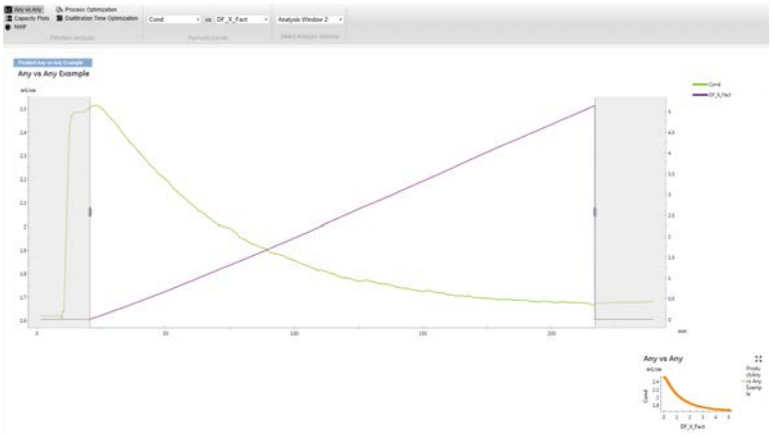


'

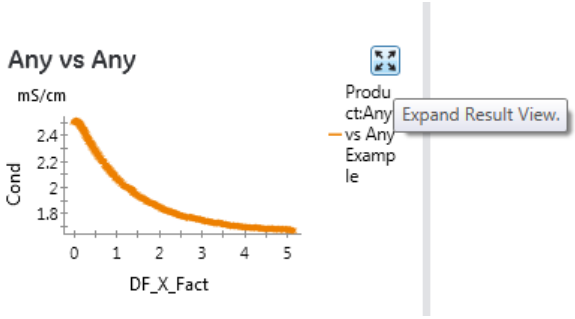


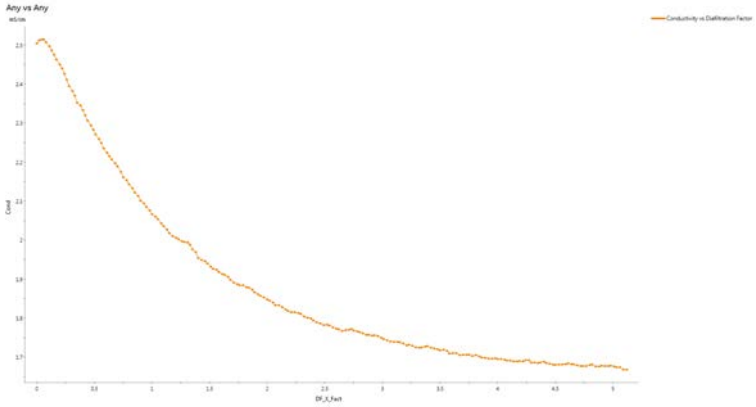
Note:

In this example, the conductivity curve will be plotted as a function of the diafiltration factor, so ***Analysis Window 2*** *from the diafiltration step has been selected.*



Step	Action
6	Click on ***Expand Result View*** to observe the conductivity plotted as a function of the diafiltration factor.





7	Rename the plot under ***Home*** →***Rename Result Curve***.

13 Feedback tuning and PID parameters

About this chapter

This chapter provides a description of the PID parameters used to control the feed retentate and permeate pressure setpoints in the ÄKTAcrossflow.

In this chapter

13.1 PID control

Whenever an automated process step requires the control of pressure (feed retentate and permeate pressure), the UNICORN control software of ÄKTAcrossflow employs PID-type controllers to control the pressure to its setpoint. Recommended default settings for the controllers (P, PI, or PID type) are listed in *table on page 259*. These settings provide fast response and robust control for most operating situations. However, the following controllers may require adjustment depending on the type and behavior of the filter:

Feed pump control

FeedPressure_PI, ***DeltaP_PI***, and ***EmptyResFeed_PI***.

These three controllers ramp the flow rate of the feed pump to achieve a desired pressure. The default settings are appropriate for flat sheet cassettes that give higher back pressure than hollow fiber cartridges at a given flow rate. When using hollow fiber cartridges, the action of the ***Integral*** controller (I parameter) can be increased (by reducing the figure for the I parameter) to yield a faster pump response and shorter ramp time, respectively. For the alternative settings, see *table on page 259*.

When creating methods with the ***Method Wizard***, the alternative settings for PID control detailed in *table on page 259* are automatically used.

TMP control

TMP_PID_PermeatePump and ***TMP_PID_RetentateControlValve***.

During TMP control, the permeate pump controls the permeate pressure by adjusting the flow rate of the permeate pump. Depending on membrane area, filter cut-off, and process conditions, the magnitude of the permeate flow rate may vary in a wide range from less than 1 mL/min to 50 mL/min and higher. Two settings are recommended to provide fast and robust control, see also *table on page 259*.

During non-product process steps when the membrane is not exposed to process fluids, thus avoiding the risk for membrane fouling or gel-layer formation, a high permeate flow rate and linear pressure-flow relationship is typical. Under these conditions, "fast" PID settings are recommended.

During non-product process steps when the membrane is not exposed to process fluids, thus with a risk of membrane fouling or gel-layer formation, slower but more robust control results in low permeate flow rates and a non-linear pressure-flow relationship. Under these conditions, “slow” PID settings are selected as default values in the strategy to provide maximum robustness of the control.

Table 13.1: Recommended settings for PID control.

Control element	PID settings	P	I	D
Feed pump control	Hollow fibers: ***FeedPressure_PI DeltaP_PI EmptyResFeed_PI*** Flat sheet cassettes: ***FeedPressure_PI DeltaP_PI EmptyResFeed_PI***	0.050.050.05 0.050.050.05	502020 150150150	N/AN/AN/A N/AN/AN/A
TMP control	***TMP_PID_RetentateControlValveTMP_PID_PermeatePump*** (membrane not exposed to proteins)***TMP_PID_PermeatePump*** (membrane exposed to proteins)***TMP_PID_PermeatePump*** (hollow fibres)	0.10.1 0.03 0.05	2020 300 75	10 0 0
PUF control	***PUF_PI_RetentateControlValve PUF_PI_PermeatePump***	0.010.001	20200	N/AN/A
Flux control	***Flux_PI_RetentateControlValve***	0.1	20	N/A
NFF control	***pNFF_PI***	0.20.05	20150	N/AN/A
Level control	***Const_RVol_P***	50	N/A	N/A

Tuning and troubleshooting of PID control

Typically, only control settings for **Feed Pump** or **Permeate Pump** need to be adjusted. The control settings for the **Retentate Control Valve** given in *table on page 259* should not be modified at all. Common methods for PID optimization (e.g., Ziegler-Nichols method) can be applied.

In most situations, good results are obtained when using the following rules of thumb:

- Slower control: increase I parameter, decrease P parameter
- Faster control: decrease I parameter, increase P parameter

No drastic changes should be applied. An appropriate measure is to change parameter I up or down by a factor of 2, while initially keeping the P parameter constant.

PI parameters for larger filter areas

When using larger filter areas, for example, > 100 cm^2, it is necessary to optimize the PI parameters.

When optimizing the parameters to obtain a faster regulation, a recommended start is given below.

- Decrease I parameter by a factor of 2
- Increase P parameter by a factor of 1.2

When the regulation is too fast, which can result in, for example, noisy curves, it may be necessary to slow down the control by doing the following.

- Increase I parameter and decrease P parameter

Hardware components using PI and PID parameters

The following table describes the PI and PID parameters that are used for hardware components and instructions.

Table 13.1: Hardware components using PI parameters.

Hardware component	Instruction
Feed pump	***Feedpump_PIDeltaP_PI***
Retentate Control Valve	***TMP_PID_RetentateControlValve Flux_PI_RetentateControlValve PUF_PI_RetentateControlValve***

Hardware component	Instruction
Permeate Pump	***TMP_PID_PermeatePump PUF_PI_PermeatePump pNFF_PI***
Reservoir	***ConstRVol_P EmptyResFeed_PI***

13.2 Description of the PI parameters and regulators

table on page 262 describes the three PID parameters used.

Table 13.1: PID parameters

Parameter	Description
P	The **P** parameter reduces the effect of an error but does not completely eliminate it. A simple P-regulator results in a stable stationary error between actual and requested flow or pressure.
I	The **I** parameter eliminates the stationary error, but results in a slight instability leading to oscillations in the actual flow or pressure. The I parameter can have values between 0 and infinity. Smaller values have a greater effect and a value of infinity has no effect. ***Note:*** *The value infinity is set as 9999 in UNICORN.*
D	In certain cases, the **D** parameter can reduce the oscillations introduced by a PI-regulator. D can have values between 0 and infinity, where larger values have a greater effect and a value of 0 has no effect. ***Note:*** *Most often, a simple PI-regulator is preferable for control of pressure, and the ÄKTAcrossflow is therefore configured by default with the D parameter set to zero.*

13.3 Setting up feedback tuning

UNICORN uses PI feedback tuning, where P and I are parameters that determine the tuning characteristics.

There are two ways to apply the feedback tuning instructions:

- In the ***Method Editor*** module.
- In the ***System Control*** module, reached with the commands ***Manual*** →***Execute Manual Instructions*** or ***System*** →***Settings***.

Instruction groups

The PI instructions are found in the following Instruction groups described in the table below.

Instruction name	Instruction group
FeedPressure_PI	System: Settings: Specials Method/Manual: Recirculation
TMP_PID_RetentateControlValve	System: Settings: Specials Method/Manual: Permeate
TMP_PID_PermeatePump	System: Settings: Specials Method/Manual: Permeate
Flux_PI_RetentateControlValve	System: Settings: Specials Method/Manual: Permeate
PUF_PI_RetentateControlValve	System: Settings: Specials Method/Manual: Permeate
PUF_PI_PermeatePump	System: Settings: Specials Method/Manual: Permeate
pNFF_PI	System: Settings: Specials Method/Manual: Permeate
DeltaP_PI	System: Settings: Specials Method/Manual: Recirculation
ConstRVol_P	System: Settings: Specials Method/Manual: Transfer
EmptyResFeed_PI	System: Settings: Specials Method/Manual: Recirculation

13.4 Optimizing the PI parameters

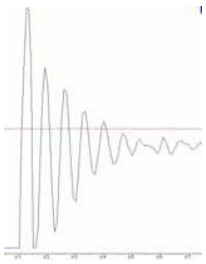
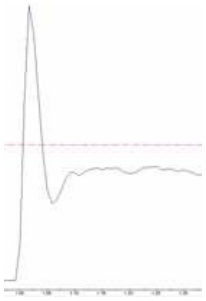
The objective to optimize the PI parameters is to obtain a sufficiently fast and smooth regulation without over-pressurization.

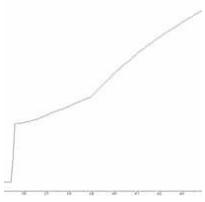
PI values that are too weak may lead to a smooth but slow ramping

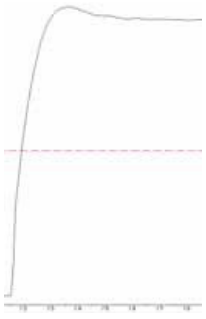
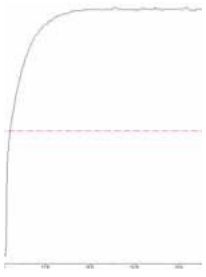
PI values that are too aggressive may lead to a fast but unstable ramping.

Example: Regulation of the Feed pump

In this example, the ***Feedpump_PI*** instruction was adjusted during manual operation of the system. The **Feed pump** was run using different PI parameter values to obtain a set Feed pressure.

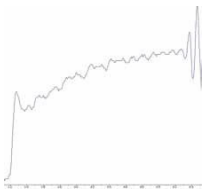
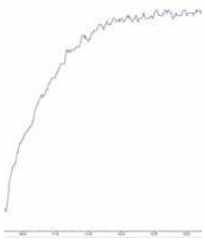
PI settings	Curve example (Feed pump flow)
1) Settings: The I parameter was set to a high value (1000), which means it has minimal effect of the regulation. The P parameter was set to a high value (1.0). Result: Uncontrolled regulation with high fluctuations.	
2) Settings: The I parameter was kept to 1000. The P parameter was set to 0.5. Result: The size of the first step is too large.	

PI settings	Curve example (Feed pump flow)
3) Settings: The I parameter was maintained at 1000. The P parameter was set to 0.2. Result: The size of the first step is still too large.	
4) Settings: The I parameter was maintained at 1000. The P parameter was set to 0.1. Result: The size of the first step is still too large.	
5) Settings: The I parameter was maintained at 1000. The P parameter was set to 0.05. Result: The size of the step seems to be correct.	
6) Settings: The P parameter is maintained at 0.05. The optimization of the I parameter starts a value of 500 and continues with 200. Result: The slope of the curve increases with decreased I values.	

PI settings	Curve example (Feed pump flow)
7) Settings: The I parameter is further decreased from 200 to 100. The P parameter is maintained at 0.05 Result: Increased slope is obtained.	
8) Settings: The P parameter was maintained at 0.05 and the I parameter was set to 25. Result: The curve indicates a too high flow before it stabilizes. The I parameter should be increased.	
9) Settings: The I parameter is increased to 50 and the P parameter is maintained at 0.05 Result: The PI parameters are optimized and a smooth and fast regulation is obtained.	

Example: Regulation of the Permeate pump

In this example, the ***TMP_PI_PermeatePump*** instruction was adjusted during manual operation of the system. TMP Control was set to 1 Bar and was monitored at different PI values.

PI settings	Curve example (TMP)
1) Settings: The P parameter was set to 1. The I parameter was set to 100. Result: Pressure peaks were observed both in the beginning of the regulation and when the TMP was reached.	
2) Settings: The P parameter was decreased to 0.2. The I parameter was decreased to 50. Result: Smooth regulation up to set the TMP and no pressure peaks were observed when the TMP was reached.	

14 Strategy instructions

About this chapter

This chapter provides details on all of the system instructions for the ÄKTAcrossflow.

In this chapter

14.1 System settings and instruction boxes

System setting: the order of instructions within each group

Alarms/ Warnings	Specials	Monitors	Curves
UVpHCond-Feed_Press Trf_Press-DeltaPTMP-Flux-ShearpNFF-ValvesFlow-pathAirSen-sorZeroLe-vel	RetentateHoldUpVolPressur-eOffsetRPCVoffset RPCVhys-teresis PPCV_SetpTo-tal_Membrane_Area Lu-men_Diameter Total_Num-ber_of_Fibers AuxOut1Aux-Out2AuxOut3AuxOut4Feed-Pressure_PI Del-taP_PITMP_PID_Retentate-ControlValve TMP_PID_Per-meatePumpFlux_ PI_Retenta-teControlValve PUF_PI_Re-tentateControlValve PUF_PI_Permeate-PumppNFF_PIEmptyRes-Feed_PI ConstRVol_PFrac-Parameters[1] Frac_Number-ing_Mode[1] Reservoir_Size	AveragingTimeUV-Pressure_Filter_Fac-tor CondTempComp-CondRefTemp pHTempCompAirSen-sorWatchPar_UV WatchPar_pH Watch-Par_CondWatch-Par_Feed_Press WatchPar_Re-ten_Press WatchPar_ PermPress Watch-Par_FeedFlow Watch-Par_Ret_Flow Watch-Par_ PermFlow WatchPar_TrfFlow WatchPar_ RetVol WatchPar_ ResVol WatchPar_ PermVol WatchPar_ TransVol WatchPar_ DeltaP WatchPar_ TMP WatchPar_ Flux WatchPar_ Shear WatchPar_ pNFF WatchPar_ConcFact WatchPar_DF_X_Fact WatchPar_ %Flux_Drop	UVCond Feed-Flow RetFlow PermFlow TrfFlow Feed_Press RetenPress PermPress pHTemp Del-taP TMP Flux Shear Con-cFact DF_X_Fct Re-tVol ResVol PermVol TransVol Con-vRatio pNFF AuxIn1 Aux-Out1

[1] Fractionation instructions are only shown when ***Fraction Collector*** has been selected in ***Administration →System Properties***.

Instruction box: the order of instructions within each group

Recirc	Permeate	Transfer	Alarms, Warnings, and Monitors	Watch[1]
FeedFlowRet-FlowFeedpres-sureDeltaP-ShearReten-tate_Valve_Block EmptyReservoir-EmptyReservoir-AbortConcentra-tion_FactorMix-erSpeedSet_Re-sVol_Totalizer-ManSample[2] FeedPres-sure_PIDel-taP_PIEmptyRes-Feed_PIRetenta-teHoldupVol	TMP_Control-Flux_ControlPer-meate_Valve_blo ckPermeate_Un-restric-ted_FlowNFF_Co nstant-FlowNFF_Con-stantPressureS-tart_Eval_Win-dowS-top_Eval_Win-dowS-et_Eval_MarkS-et_PermVol_To-taliz-er %Flux_Drop_Cal cTotal_Mem-brane_Sur-face_AreaLu-men_Diameter-vTotal_Num-ber_Of_Fi-bersTMP_PI_Re-tentateControl-ValveTMP_PIPer-meatePump-Flux_PI_Retenta-teControlValve-PUF_PI_Retenta-teControlValve-PUF_PI_Permea-tePumppNFF_PI-	Trans_Flow-Con-stant_Ret_Volu-meTrans-fer_Valve_Block-sTrans-fer_Purge_Valve-Set_TrfVol_Total-izerDF_Exchan-geFactorMe-thod_Base[1] Con-stRVol_P	AutoZeroUVAvera-ginfTimeUVPres-sure_Filter_FactorA-larm_UVAlarm_pHA-larm_CondA-larm_FeedPressA-larm_TrfPressA-larm_DeltaPA-larm_TMPAlarm_Flux-Alarm_pNFFA-larm_ShearA-larm_ValvesA-larm_FlowpathA-larm_AirSensorA-larm_ZeroLevel-WatchPar_UVWatch-par_pHWatch-Par_CondWatch-Par_FeedPressWatch-Par_RetenPress-WatchPar_Perm-PressWatchPar_Feed-FlowWatchPar_Re-ten_FlowWatch-Par_PermFlowWatch-Par_TrfFlowWatch-par_ResVolWatch-par_RetVolWatch-par_PermVolWatch-par_TransfVolWatch-par_DeltaPWatch-par_TMPWatch-par_FluxWatch-par_ShearWatch-par_pNFFWatch-par_ConcFactor-	Hold_Until-Watch_UV-Watch_pHWatch _Cond-Watch_Feed-PressWatch_Re-tenPress-Watch_Perm-Press-Watch_Feed-FlowWatch_Re-ten_Flow-Watch_Perm-Flow-Watch_TrfFlow-Watch_RetVol-Watch_ResVol-Watch_PermVol-Watch_Trans-fVolWatch_Del-taP-Watch_TMPWat ch_Flux-Watch_Shear-Watch_pNFFWa tch_ConcFact-Watch_DF_X_Fa ctWatch_Flux_Dr opWatch_Air-Sensor-Watch_ZeroLe-velWatch_Aux-In1Watch_Aux-In2Watch_Aux-In3Watch_Aux-In4Watch_Off

Recirc	Permeate	Transfer	Alarms, Warnings, and Monitors	Watch[1]
	Fractionation[3] Fractionation-Stop[3] ResetTubeNumberFeedTube[3]		Watch-par_DF_X_FctWatch-par_%Flux_DropUV-lampOFFLevelSensor-Calibration	

[1] Only in Method Editor.

[2] Only in System control.

[3] Fractionation instructions are only shown when ***Fraction Collector*** has been selected in ***Administration*** →***System Properties***.

14.2 Recirculation instructions

FeedFlow

Instruction name***Feed-Flow***	Formula**Qf**	Group***Method/Manual →Recirc***
Parameter 1 name***Flow-Rate***	Mode**Control Mode Direct**	Position name (default underlined)0.0 mL/min (0-600)
Instruction Help textStarts the flow on the ***Feed Pump***. Feed Flow should stabilize at 98% of set value before a new instruction is executed. To stall a new instruction until the ***DeltaP*** has stabilized, a ***Watch_Stable Signal DeltaP*** should be programmed in the methods. Instruction resets ***RetFlow***, ***Feed-Pressure***, ***DeltaP***, and ***Shear***.		

RetFlow

Instruction name***Ret-Flow***	Formula**Qr = Qf-Qp**	Group***Method/Manual →Recirc***
Parameter 1 name***Flow-Rate***	Mode**Control Mode Direct**	Position name (default underlined)0.0 mL/min (0-600)
Instruction Help textStarts the flow on the ***Feed Pump***. ***RetFlow*** should stabilize at 98% of the set value before a new instruction is executed. Instruction resets ***FeedFlow***, ***FeedPressure***, ***DeltaP***, and ***Shear***. To stall a new instruction until the ***DeltaP*** has stabilized, a ***Watch_Stable Signal DeltaP*** should be programmed in the methods. Instruction resets ***FeedFlow***, ***FeedPressure***, ***DeltaP***, and ***Shear***.		

FeedPressure

Instruction name***Feed-Pressure***	Formula**Pf**	Group***Method/Manual →Recirc***

Parameter 1 name***Pressure***	Mode**Control Mode Direct**	Position name (default underlined)<u>0.00</u> bar (0.00 - 5.20)
Instruction Help textStarts the flow on the ***Feed Pump*** until the ***Feed Pressure*** setpoint is reached. The ***FeedPressure*** should stabilize at 98% of the set value before a new instruction is executed. To stall a new instruction until the ***DeltaP*** has stabilized, a ***Watch_Stable Signal DeltaP*** should be programmed in the methods. Instruction resets ***FeedFlow***, ***RetFlow***, ***DeltaP***, and ***Shear***.		

DeltaP

Instruction name***DeltaP***	Formula**DeltaP = Pf - Pr**	Group***Method/Manual →Recirc***
Parameter 1 name***Pressure***	Mode**Control Mode PID**	Position name (default underlined)<u>0.00</u> bar (0.00 - 5.20)
Instruction Help textStarts the flow on the ***Feed Pump*** until the ***Pf-Pr*** reaches the ***DeltaP*** setpoint. The ***DeltaP*** should stabilize at 98% of the set value before a new instruction is executed. To stall a new instruction until the ***DeltaP*** has stabilized, a ***Watch_Stable Signal DeltaP*** should be programmed in the methods. Instruction resets ***FeedFlow***, ***RetFlow***, ***Shear***, and ***FeedPressure***.		

Shear

Instruction name ***Shear***	Formula **Shear = (4 × Qf)/(No. of Fibers × π × (fiberradius)^3)**		Group ***Method/Manual →Recirc***
Parameter 1 name***FlowRate***	Mode**Control Mode PID**	***Note:*** Only for hollow fibers	Position name (default underlined) <u>0</u> s^{-1} (0 - 20 000)

Instruction Help text This instruction is used for hollow fiber cartridges only and starts the flow on the ***Feed Pump*** until the flow rate that equals a shear rate for a particular hollow fiber cartridge is reached (which is dependent upon the fiber lumen diameter and number of individual fibers contained within the cartridge). The ***Shear*** should stabilize at 98% of the set value before a new instruction is executed. To stall a new instruction until the ***DeltaP*** has stabilized, a ***Watch_Stable Signal DeltaP*** should be programmed in the methods. Instruction resets ***FeedFlow***, ***RetFlow***, ***FeedPressure***, and ***DeltaP***.

Retentate_Valve_Block

Instruction name***Retentate_Valve_Block***	Group***Method/Manual →Recirc***
Parameter name***Macro***	Position name (default underlined)R-VB_Recycle, R-VB-Out1, R-VB-Out2, R-VB-Out3
Instruction Help Selects the position for the ***Retentate Valve Block***, either ***R-VB-Recycle***, ***R-VB-Out1*** (port located prior to filter device), ***R-VB-Out2***, or ***R-VB-Out3***. When ***Feed-Flow*** > 0 or ***Constant_Retentate_Volume*** is active, no action is allowed. A warning will be raised. Alarm help text 1: ***Retentate_Valve_Block*** instruction not allowed during ***Constant_Retentate_Volume*** Alarm help text 2: ***Retentate_Valve_Block*** instruction not allowed when ***Feed setpoint*** > 0 Alarm help text 3: ***Retentate_Valve_Block*** instruction not allowed during ***EmptyReservoir*** Alarm help text 4: ***Retentate_Valve_Block*** instruction not allowed during ***NFF***	

RPCVoffset

Instruction name***RPCVoffset***	Group***System →Settings →Specials***
Parameter name***Pressure***	Position name (default underlined)350, (0 - 999)

Instruction Help Sets offset on ***Retentate Control Valve*** used by system during ***TMP_Control***, ***Flux_Control***, and ***Permeate_Unrestricted_Flow***

Reservoir_Size

Instruction name***Reservoir_Size***	Group***System*** →***Settings*** →***Specials***
Parameter 1 name***Size***	Position name (default underlined)350 mL, (350, 1100 mL)
Instruction Help Sets reservoir size to 350 or 1100 mL.	

EmptyReservoir

Instruction name***EmptyReservoir***	Group***Method/Manual*** →***Recirc***
Parameter 1 name***RetValveOutlet*** Parameter 2 name***MaxFeedPressure*** Parameter 2 name***MaxFeedFlow***	Position name (default underlined)R-VB-Out1, R-VB-Out2, R-VB-Out3 0.000 bar (0.00 - 5.20 bar) 600 mL/min (0 - 600 mL/min)
Instruction Help Instruction uses ***R-VB-Out*** to open a retentate outlet and ***FeedPump*** to empty the reservoir in a controlled manner. In order to perform this, the ***FeedFlow*** is reduced when the reservoir level is below a certain value and ***R-VB-Out*** creates a back pressure of approximately 2 bar to avoid siphoning. When the zero level is reached, defined as just below reservoir bottom surface, ***FeedFlow*** is immediately stopped.	

EmptyReservoirAbort

Instruction name***EmptyReservoirAbort***	Group***Method/Manual*** →***Recirc***

Parameter 1 name***RetValvePort*** Parameter 2 name***FeedFlow***	Position name (default underlined)R-VB-Recycle 0.000 bar (0.00 - 5.20 bar) 0.0 mL/min (0.0 - 600.0 mL/min)
Instruction Help ***EmptyReservoirAbort*** aborts the sequence initiated by the ***EmptyReservoir*** instruction. Instruction immediately stops the ***FeedFlow***, and sets ***R-VB-Out*** to the ***Recycle*** position. The ***Watch EmptyResFinished*** signal is then generated.	

Concentration_Factor

Instruction name***Concentration_Factor***	Group***Method/Manual →Recirc***
Parameter 1 name***Mode*** Parameter 2 name***Type***	Position name (default underlined)Off, On FedBatch, TankBatch
Instruction Help Starts the ***Concentration Factor*** calculation. Use a watch command in the method to set the endpoint on concentration factor. ***FedBatch*** cannot be operated without a ***Set_ResVol_Totalizer*** volume entered. Detailed information can be found in User Manual.	

MixerSpeed

Instruction name***MixerSpeed***	Group***Method/Manual →Recirc***
Parameter 1 name***Speed***	Position name (default underlined)Auto (0 - 600 rpm)
Instruction Help Sets the reservoir mixer speed. ***Auto*** adjusts the mixer speed to the reservoir size (set in ***System →Settings***) and linearly adjusts the speed to the current ***ResVol***. Minimum speed is 80 rpm; maximum speed is 200 rpm for the 350 mL reservoir and 300 rpm for the 1100 mL reservoir.	

Set_ResVol_Totalizer

Instruction name***Set_ResVol_Totalizer***	Group***Method/Manual →Recirc***
Parameter 1 name***Speed***	Position name (default underlined)<u>0.0</u> mL (0.00 - 5 000.00)
Instruction Help Sets a desired volume for the reservoir volume totalizer. The totalizer value is calculated based on pump actions. Flow from the ***Transfer Pump*** will increase the value, if it is directed to the reservoir. Permeate flow measured by the ***Permeate Pump***, if a permeate outlet valve is open, will decrease the volume, but not if the ***Permeate_Valve_Block*** is in the ***Recycle*** position and the ***Transfer_Purge_Valve*** is in position ***Reservoir***. ***FeedFlow*** with an open retentate valve position will also decrease the totalizer volume.	

ManSample

Instruction name***ManSample***	Group***Manual →Recirc***
Parameter 1 name***Volume***	Position name (default underlined)<u>0.00</u> mL (0.00 - 100.00)
Instruction Help Reduces reservoir volume totalizer with the volume of removed sample at the moment of execution of the instruction. For more information, see *Section 10.4 Manual sampling during the run, on page 216*. For correct concentration and diafiltration factor calculations, it is important that the ***ManSample*** instruction is executed immediately after taking the sample from the reservoir.	

FeedPressure_PI

Instruction name***FeedPressure_PI***	Group***System:Settings:SpecialsMethod/Manual: Recirc***
Parameter 1 name***P*** parameter 2 name***I***	Position name (default underlined)<u>0.05</u> (0.00 - 10 000.00) <u>150.00</u> sec (0.00 - 10 000.00

Instruction Help These parameters are used to tune the feedback control when ***FeedPressure-control*** is active.

DeltaP_PI

Instruction name***DeltaP_PI***	Group***System:Settings:SpecialsMethod/Manual: Recirc***
Parameter 1 name***P*** Parameter 2 name***I***	Position name (default underlined)<u>0.05</u> (0.00 - 10 000.00) <u>150.00</u> sec (0.00 - 10 000.00
Instruction Help These parameters are used to tune the feedback control during ***DeltaP***. For more information, see *Chapter 13 Feedback tuning and PID parameters, on page 257*.	

EmptyResFeed_PI

Instruction name***EmptyResFeed_PI***	Group***System:Settings:SpecialsMethod/Manual: Recirc***
Parameter 1 name***P*** Parameter 2 name***I***	Position name (default underlined)<u>0.05</u> (0.00 - 10 000.00) <u>150.00</u> sec (0.00 - 10 000.00
Instruction Help These parameters are used to tune the feedback control during ***EmptyReservoir***. For more information, see *Chapter 13 Feedback tuning and PID parameters, on page 257*.	

RetentateHoldupVol

Instruction name***RetentateHoldup-Vol***	Group***System:Settings:SpecialsMethod/Manual: Recirc***

Parameter name***Volume***	Position name (default underlined)<u>18.20</u> mL (0.00 - 100.00)
Instruction Help See *Section A Membrane and cartridge selection, on page 333* for more information.	

14.3 Permeate instructions

TMP_Control

Instruction name***TMP_Control***	Formula**TMP = ((Pf + Pr/2)-Pp)**	Group***Method/Manual →Permeate***
Parameter 1 name***Flow-Rate***	Mode**Control Mode PID** ***Note:*** *No overshooting (max 10% of setpoint)*	Position name (default underlined)<u>0.00</u> bar (0.00 - 5.20)
Instruction Help:Adjusts the ***Retentate_Control_Valve*** and the ***Permeate Pump*** to maintain a ***TMP*** setpoint. Prior to activation of ***TMP control***, make sure that ***DeltaP*** is stable. Resets, ***Flux_Control*** and ***Permeate_Unrestricted_Flow***.		

Flux_Control

Instruction name***Flux_Control***	Formula**Flux = (Qp[l/h])/(A[m^2])**	Group***Method/Manual →Permeate***
Parameter 1 name***Flux*** Parameter 2 name***TMPlimit***	Mode**Control Mode PID** ***Note:*** *Pp ≤ Pr*	Position name (default underlined)<u>0.0</u> LMH (0.0 - 4800.0) <u>Off</u> (0.01 - 5.20 bar)
Instruction Help: Starts the flow on the ***Permeate Pump*** as a flux rate. If the permeate pressure is < 0.2 bar, the ***Retentate Control Valve*** will lift the permeate pressure to the offset value. When the ***TMPlimit*** is activated, the system will pause the run if the setpoint is reached. Resets ***TMP_Control*** and ***Permeate_Unrestricted_Flow***. The ***Retentate Control Valve*** will lift the permeate pressure to 95% of the offset value before the flux rate is ramped up in a 30 second interval.		

Permeate_Valve_Blocks

Instruction name***Permeate_Valve_Block***	Group***Method/Manual*** →***Permeate***
Parameter 1 name***Macro***	Position name (default underlined)Closed, ***P-VB-Recycle***, ***P-VB-Out1***, ***P-VB-Out2***, ***P-VB-Out3***
Instruction Help Selects the position for the ***Permeate_Valve_Block*** to ***P-VB-Recycle***, ***P-VB-Out1***, ***P-VB-Out2***, or ***P-VB-Out3***. No valve block action allowed when ***P-VB-recycle*** is used and a permeate control mode is active. A warning will be raised. During ***Constant_Retentate_Volume***, the ***P-VB-recycle*** position is not allowed. A warning will be raised. It is possible to change the ***Permeate_Valve_Block*** position between ***P-VB-Out1***, ***PVB-Out2***, and ***P-VB-Out3*** when the permeate flow is greater than 0. When opening a new valve outlet, the old outlet will remain open for approximately one second before closing. ***Permeate_Valve_Block*** will remain in position at ***Pause*** and the flow on the ***Permeate Pump*** goes down to 0 mL/min.	
Warning Help text 1 Instruction ignored. No valve block action allowed when **P-VB-recycle** is used and permeate control mode is active.	
Warning help text 2 Instruction ignored. **P-VB-recycle** is not allowed during ***Constant_retentate_volume***.	

Permeate_Unrestricted_Flow

Instruction name***Permeate_Unrestricted_Flow***	Formula**Pp**	Group***Method/Manual*** →***Permeate***
Parameter 1 name***Pressure***	Mode**Control Mode PID** ***Note:*** *Pp ≤ Pr*	Position name (default underlined)Disabled, Enabled

Instruction Help text: Starts the flow on the ***Permeate Pump*** at the offset permeate pressure. If ***Pr*** > ***Pp***, the ***Pr*** value is used as the offset ***Pp***. The ***Retentate Control Valve*** lifts ***Pr*** to the offset ***Pp*** if ***Pr*** < offset. Resets ***TMP_Control*** and ***Flux_Control***.

Normal_Flow_Filtration

NFF_ConstantFlow

Instruction name***NFF_ConstantFlow***	Formula**Qf**	Group***Method/Manual →Permeate***
Parameter 1 name***Flow***	Mode**Control Mode Direct**	Position name (default underlined)Off 0.0 mL/min (0.0 - 600.0)
Instruction Help text:Starts the ***Feed Pump*** at the constant feed flow setpoint and the ***Permeate Pump*** at 20% of the constant feed flow setpoint. The ***Retentate Pressure Control Valve*** is closed to close the retentate loop. The ***Permeate Pressure Control Valve*** is opened completely. Instruction ignored if the feed flow > 0 mL/min		

NFF_ConstantPressure

Instruction name***NFF_ConstantPressure***	Formula**Pf**	Group***Method/Manual →Permeate***
Parameter 1 name***Pressure***	Mode**Control Mode PID**	Position name (default underlined)Off 0.0 bar (0.00 - 5.20)
Instruction Help text:Starts the flow on the ***Feed Pump*** to run at the chosen constant pressure setpoint and starts the ***Permeate Pump*** at the offset permeate pressure. If ***Pr*** > ***Pp***, the ***Pr*** value is used as the offset. The ***Retentate Control Valve*** lifts the ***Pr*** to the offset if ***Pr*** < offset. Resets ***TMP_Control*** and ***Flux_Control***.		

PressureOffset

Instruction name***PressureOffset***	Group***System →Settings →Specials***

Parameter name***Pressure***	Set point (default underlined)0.2 bar (0.2 - 1.0 bar)
Instruction Help text:Sets the permeate offset pressure used by system during ***TMP_Control***, ***Flux_Control*** and ***Permeate_Unrestricted_Flow***.	

Evaluation Instructions

Start_Eval_Window

Instruction name***Start_Eval_Window***	Group***Method/Manual →Permeate***
Parameter name***Mode***	Position name (default underlined)0 = Any vs. any, 1 = Capacity, 2 = DFTimeOpt
Instruction Help text:Starts collection of chosen sets of data for the ***Filtration Analysis*** tool in ***Evaluation***. Several sets of data Windows can be defined during a run. For more information, see *Chapter 12 Evaluating ÄKTAcrossflow results using Filtration Analysis, on page 227*.	

Stop_Eval_Window

Instruction name***Stop_Eval_Window***	Group***Method/Manual →Permeate***
Parameter name***Mode***	Position name (default underlined)0 = Any vs. any, 1 = Capacity, 2 = DFTimeOpt
Instruction Help text:Stops collection of chosen sets of data for the ***Filtration Analysis*** tool in ***Evaluation***. Several sets of data Windows can be defined during a run. For more information, see *Chapter 12 Evaluating ÄKTAcrossflow results using Filtration Analysis, on page 227*.	

Set_Eval_Mark

Instruction name***Set_Eval_Mark***	Group***Method/Manual →Permeate***
Parameter name***Mode***	Position name (default underlined)0 = ProcessOptimization, 1 = ExtData_vs_Capacity, 2 = NormalisedWaterFlux

Instruction Help text:Sets a mark for data collection for the ***Filtration Analysis*** tool in ***Evaluation***. Several ***Mark*** instructions can be defined during a run.

Set_PermVol_Totalizer

Instruction name***Set_PermVol_Totalizer***	Group***Method/Manual*** →***Permeate***
Parameter name***Volume***	Set point (default underlined)<u>0.00</u> mL (0.00 - 5 000.00)
Instruction Help text:Sets a desired volume for the permeate volume totalizer. The totalizer value is calculated based on the action of the ***Permeate Pump***. The totalizer volume increases with permeate flow.	

%Flux_Drop_Calculation

Instruction name***%Flux_Drop_Calculation***	Group***Method/Manual*** →***Permeate***
Parameter name***Mode***	Position name (default underlined)<u>Off</u>, On
Instruction Help text:Upon activation, this instruction latches onto the current permeate flux rate and sets it as the reference value. The instruction calculates the % drop of the current flux value based on the reference value. A increase in flux values does not register and the ***%Flux_Drop_Calculation*** value will remain at 0.	

Total_Membrane_Surface_Area

Instruction name***Total_Membrane_Surface_Area***	Group***Method/Manual*** →***Permeate System*** →***Settings*** →***Specials***
Parameter name***Area***	Position name (default underlined)<u>50</u> cm^2 (1 - 1 200 cm^2)
Instruction Help text:Used in the calculation of the permeate flux value with the equation: Flux=(Qp[l/h])/(A[m]).	

Lumen_Diameter

Instruction name***Lumen_Diameter***	Group***Method/Manual*** →***Permeate System*** →***Settings*** →***Specials***
Parameter name***Diameter***	Set point (default underlined)1.00 mm (0.1 - 10.0)
Instruction Help text:Together with ***Total_Number_Of_Fibers***, the ***Shear*** rate can be calculated with the formula Shear = (4 × Qf) / (number of fibers × π × (fiber-radius)^3)	

Total_Number_of_Fibers

Instruction name***Total_Number_of_Fibers***	Group***Method/Manual*** →***Permeate System*** →***Settings*** →***Specials***
Parameter name***Quantity***	Position name (default underlined)1 (1 - 1 000)
Instruction Help text:Together with the ***Lumen_Diameter***, the ***Shear*** rate can be calculated with the formula Shear = (4 × Qf) / (number of fibers × π × (fiberradius)^3)	

PPCV_Setp

Instruction name***PPCV_Setp***	Group***System*** →***Settings*** →***Specials***
Parameter name***Pressure***	Set point (default underlined)700 (0 - 999)
Instruction Help text:Sets the offset on the ***Permeate Control Valve*** used by system during ***TMP_Control***, ***Flux_Control*** and ***Permeate_Unrestricted_Flow*** .	

TMP_PI_RetentateControlValve

Instruction name***TMP_PI_RetentateControlValve***	Group ***Method/Manual*** →***Permeate System*** →***Settings*** →***Specials***

Parameter 1 name***P*** Parameter 2 name***I*** Parameter 3 name***D***	Setpoint name (default underlined)<u>0.10</u> (0.00 - 10 000.00) <u>20.00</u> sec (0.00 - 10 000.00) <u>1.00</u> sec (0.00 - 10 000.00)
Instruction Help text:These parameters are used to tune the feedback control when ***TMP_Control*** is active. For details, see *Chapter 13 Feedback tuning and PID parameters, on page 257* .	

Flux_PI_RetentateControlValve

Instruction name***Flux_PI_Retentate-ControlValve***	Group***Method/Manual →Permeate System →Settings →Specials***
Parameter 1 name***P*** Parameter 2 name***I***	Setpoint name (default underlined)<u>0.10</u> (0.00 - 10 000.00) <u>20.00</u> sec (0.00 - 10 000.00)
Instruction Help text:These parameters are used to tune the feedback control when ***Flux_Control*** is active. For details, see *Chapter 13 Feedback tuning and PID parameters, on page 257*.	

PUF_PI_RetentateControlValve

Instruction name***PUF_PI_Retentate-ControlValve***	Group***Method/Manual →Permeate System →Settings →Specials***
Parameter 1 name***P*** Parameter 2 name***I***	Setpoint name (default underlined)<u>0.10</u> (0.00 - 10 000.00) <u>20.00</u> sec (0.00 - 10 000.00)
Instruction Help text:These parameters are used to tune the feedback control when ***Permeate_Unrestricted_Flow*** is active. For details, see *Chapter 13 Feedback tuning and PID parameters, on page 257*.	

PUF_PI_PermeatePump

Instruction name***PUF_PI_Permeate-Pump***	Group***Method/Manual →Permeate System →Settings →Specials***
Parameter 1 name***P*** Parameter 2 name***I***	Setpoint name (default under-lined)<u>0.001</u> (0.00 - 10 000.00) <u>200.00</u> sec (0.00 - 10 000.00)
Instruction Help text:These parameters are used to tune the feedback control when ***Permeate_Unrestricted_Flow*** is active. For details, see *Chapter 13 Feed-back tuning and PID parameters, on page 257*.	

pNFF_PI

Instruction name***pNFF_PI***	Group***Method/Manual →Permeate Sys-tem →Settings →Specials***
Parameter 1 name***P*** Parameter 2 name***I***	Setpoint name (default underlined)<u>0.050</u> (0.000 - 10 000.000) <u>150.00</u> sec (0.000 - 10 000.000)
Instruction Help text:These parameters are used to tune the feedback control when ***Normal_Flow_Filtration*** is active. For details, see *Chapter 13 Feedback tuning and PID parameters, on page 257*.	

Fractionation

Instruction name***Fractionation***	Group***Method/Manual →Permeate***
Parameter name***FracSize***	Setpoint name (default underlined)<u>0.0</u> mL (0.0 - 50.0)
Instruction Help text:Starts fraction collection if the fraction size specified by the ***FracSize*** parameter is > 0 mL. The tube change is delayed by the delay volume specified in ***System →Settings →Specials →FracParameters***. The fractionation is stopped with the instruction ***FractionationStop***.	

FeedTube

Instruction name***FeedTube***	Group***Method/Manual*** →***Permeate***
Instruction Help text:During fractionation, the ***FeedTube*** instruction moves the tube rack forward one tube after the delay volume specified in ***System*** →***Settings*** →***Specials*** →***FracParameters*** →***DelayVol*** has been collected and a fraction mark is given. When Fractionation is not used, ***FeedTube*** moves the rack instantly and no fraction mark is given.	

FractionationStop

Instruction name***FractionationStop***	Group***Method/Manual*** →***Permeate***
Instruction Help text:Stops the fraction collection after the delay volume specified in ***System*** →***Settings*** →***Specials*** →***FracParameters*** →***DelayVol*** has been collected.	

ResetFracNumber

Instruction name***ResetFracNumber***	Group***Method/Manual*** →***Permeate***
Warning Help: ***ResetFracNumber*** is not allowed during fractionation. Instruction ignored.	

14.4 Transfer instructions

Transfer Flow

Instruction name***Transfer Flow***	Group***Method/Manual*** →***Transfer***
Parameter name***FlowRate***	Position name (default underlined)<u>0.0</u> mL (0.0 - 200.0)
Instruction Help text:Starts the flow on the **Transfer pump** at the set flow rate.	

Constant_Retentate_Volume

Instruction name***Constant_Retentate_Volume***	Formula: **Qt = Qp**	Group***Method/Manual*** →***Transfer***
Parameter name***Mode***	Mode: **Control Mode**	Position name (default underlined)<u>CRV_Off</u>, CRV_On
CRV_On = liquid level in the reservoir is kept constant at the level detected when the instruction is activated by adjusting the transfer flow to compensate for detected changes in the reservoir level. ***CRV_Off*** = immediate inactivation of the instruction. If the retentate valve is open when executing ***Constant_Retentate_Volume***, a warning will be raised and the instruction will be ignored. If **P-VB-recycle** is open when executing ***Constant_Retentate_Volume CRV_On***, a warning will be raised and the instruction ignored		
Warning Help text 1 ***Constant_Retentate_Volume*** is not allowed with **Retentate_Valve_Block** open. Instruction ignored. ***Warning Help*** text 2 ***Constant_Retentate_Volume*** is not allowed with **P-VB-recycle** position open. Instruction ignored.		

Transfer_Valve_Blocks

Instruction name***Transfer_Valve_Blocks***	Group***Method/Manual*** →***Transfer***

Parameter name***Macro***	Position name (default underlined)Closed, T-VB-In1, T-VB-In2, T-VB-In3, T-VB-In4, T-VB-In5, T-VB-In6, T-VB-In7, T-VB-In8
Instruction Help text:Selects the position for the ***Transfer_Valve_Blocks***, ***T-VB-In1***, ***T-VB- In2***, ***T-VB- In3***, ***T-VB- In4***, ***T-VB- In5***, ***T-VB- In6***, ***T-VB- In7***, ***T-VB- In8*** The inlet valves will not be set to their default position (***Closed***) at ***End*** until the flow on the pump has gone down to 0 mL/min.	

Transfer_Purge_Valve

Instruction name***Transfer_Purge_Valve***	Group***Method/Manual*** →***Transfer***
Parameter name ***Instruction***	Position name (default underlined) To_Reservoir, Waste
Instruction Help text: Selects transfer flow direction to ***Reservoir*** or ***Waste***. The ***Waste*** position is useful when cleaning the transfer inlets .	

Set_TrfVol_Totalizer

Instruction name***Set_TrfVol_Totalizer***	Group***Method/Manual*** →***Transfer***
Parameter name***Volume***	Position name (default underlined)0.00 mL (0.00 - 5 000.00)
Instruction Help text:Sets a desired volume for the transfer volume totalizer. The totalizer value is calculated based on the action of the ***Transfer Pump***. The totalizer volume increases with transfer flow.	

DF_Exchange_Factor

Instruction name***DF_Exchange_Factor***	Group***Method/Manual*** →***Transfer***

Parameter name***Mode***	Position name (default underlined)Off, On
Instruction Help text:The ***DiaFiltration Exchange Factor*** calculates the relationship between the total buffer volume introduced into the reservoir during a diafiltration step and the retentate volume when the diafiltration was started.	

MethodBase

Instruction name***MethodBase***	Group***Method →Transfer***
Parameter name***Base***	Position name (default underlined)PermeatePump, FeedPump, TransferPump
Instruction Help:Sets the base for method volume calculation to the chosen pump.	

ConstRVol_P

Instruction name***ConstRVol_P***	Group***Method/Manual →Transfer System →Settings →Specials***
Parameter name***P***	Position name (default underlined)50.000 mbar, (0.000 - 10 000.000)
Instruction Help:The P parameter affects the response of the CRV algorithm. A higher value causes faster transfer flow increase to compensate for a decreasing reservoir level, and vice versa. The ***P*** parameter also affects how quickly the start level is restored after the CRV instruction is turned off. Note: A higher ***P*** parameter value amplifies the noise in the level sensor signal, which may cause instability. For details, see *Chapter 13 Feedback tuning and PID parameters, on page 257*.	

14.5 Alarms, Warnings, and Monitors

AutozeroUV

Instruction name***AutoZeroUV***	Group***Method/Manual →Alarms&Monitors***
Instruction Help:Sets the relative AU to zero.	

AveragingTime

Instruction name***AveragingTimeUV***	Group***System:Settings:MonitorsMethod/Manual:Alarms&Monitors***
Parameter name***AvTimeUV***	Position name (default underlined)0.02, 0.04, 0.08, 0.16, 0.32, 0.64, 1.30, 2.60, 5.10, 10
Instruction Help:Filters the noise in the UV signal. Averaging time is the time interval used for calculating the moving average of the absorbance signal. A long averaging time will smooth out noise efficiently, but will also distort the peaks.	

Pressure_Filter_Factor

Instruction name***Pressure_Filter_Factor***	Group***System:Settings:MonitorsMethod/Manual:Alarms&Monitors***
Parameter name***AvTimeUV***	Position name (default underlined)30 (NoFilter, 2–100)
Instruction Help:When active all pressure signals are filtered to avoid overshooting and hysteresis during pressure regulating operations, i.e., all control modes.	

Alarm_UV

Instruction name***Alarm_UV***	Group***System:Settings:Alarms Method/Manual:Alarms&Monitors***

Parameter 1 name***Mode*** Parameter 2 name***High Alarm*** Parameter 3 name***Low Alarm***	Position name (default underlined)Disabled, Enabled Setpoint (default underlined)6000.000 mAU (-6000.000–6000.000) Setpoint (default underlined)-6000.000 mAU (-6000.000–6000.000)
Instruction Help:Sets the alarm limits for the UV signal.	

Alarm_pH

Instruction name***Alarm_pH***	Group***System:Settings:Alarms Method/Manual:Alarms&Monitors***
Parameter 1 name***Mode*** Parameter 2 name***High Alarm*** Parameter 3 name***Low Alarm***	Position name (default underlined)Disabled, Enabled Setpoint (default underlined)14.00 pH (0.00–14.00) Setpoint (default underlined)0.00 pH (0.00–14.00)
Instruction Help:Sets the alarm limits for the pH signal.	

Alarm_Cond

Instruction name***Alarm_Cond***	Group***System:Settings:Alarms Method/Manual:Alarms&Monitors***
Parameter 1 name***Mode*** Parameter 2 name***High Alarm*** Parameter 3 name***Low Alarm***	Position name (default underlined)Disabled, Enabled Setpoint (default underlined)999.99 mS/cm (0.00–1000.00) Setpoint (default underlined)0.00 mS/cm (0.00–1000.00)
Instruction Help:Sets the alarm limits for the conductivity signal.	

Alarm_FeedPress

Instruction name***Alarm_FeedPress***	Group***System:Settings:Alarms Method/Manual:Alarms&Monitors***
Parameter 1 name***Mode*** Parameter 2 name***High Alarm*** Parameter 3 name***Low Alarm***	Position name (default underlined)Disabled, Enabled Setpoint (default underlined)5.20 bar (0.00–5.20) Setpoint (default underlined)0.00 bar (0.00–5.20)
Instruction Help:Sets the alarm limits for pressure from the feed pump. When reached, the system is set to ***Pause***	
Alarm Help: ***HighAlarm***: The feed pressure has exceeded the ***HighAlarm*** limit ***LowAlarm***: The feed pressure has fallen below the ***LowAlarm*** limit.Pressure limits are set by ***AlarmFeed_Press***.	

Alarm_TrfPress

Instruction name***Alarm_TrfPress***	Group***System:Settings:Alarms Method/Manual:Alarms&Monitors***
Parameter 1 name***Mode*** Parameter 2 name***High Alarm*** Parameter 3 name***Low Alarm***	Position name (default underlined)Disabled, Enabled Setpoint (default underlined)5.20 bar (0.00–5.20) Setpoint (default underlined)0.00 bar (0.00–5.20)
Instruction Help:Sets the alarm limits for pressure from the transfer pump. When reached, the system is set to ***Pause.***	
Alarm Help: ***HighAlarm***: The transfer pressure has fallen below the ***HighAlarm*** limit. ***LowAlarm***: The transfer pressure has fallen below the ***LowAlarm*** limit Pressure limits are set by ***AlarmFeed_Press***.Press ***Acknowledge*** on the error message window, then press ***Continue***.	

Alarm_DeltaP

Instruction name***Alarm_DeltaP***	Group***System:Settings:Alarms Method/Manual:Alarms&Monitors***
Parameter 1 name***Mode*** Parameter 2 name***High Alarm*** Parameter 3 name***Low Alarm***	Position name (default underlined)Disabled, Enabled Setpoint (default underlined)5.20 bar (0.00–5.20) Setpoint (default underlined)0.00 bar (0.00–5.20)
Instruction Help:Sets the alarm limits for the ***DeltaP*** (***Pf-Pr***). When reached, the system is set to ***Pause***.	
Alarm Help: ***HighAlarm*** cause: the ***DeltaP*** has exceeded the ***HighAlarm*** limit.***LowAlarm*** cause: the ***DeltaP*** has fallen below the ***LowAlarm*** limit.Pressure limits are set by ***Alarm_DeltaP*** .	

Alarm_TMP

Instruction name***Alarm_TMP***	Group***System:Settings:Alarms Method/Manual:Alarms&Monitors***
Parameter 1 name***Mode*** Parameter 2 name***High Alarm*** Parameter 3 name***Low Alarm***	Position name (default underlined)Disabled, Enabled Setpoint (default underlined)5.20 bar (0.00-5.20) Setpoint (default underlined)0.00 bar (0.00-5.20)
Instruction Help:Sets the alarm limits for transmembrane pressure (***TMP***). When reached the system is set to ***Pause***.	
Alarm Help text: ***HighAlarm*** cause: The ***TMP*** has exceeded the ***HighAlarm*** limit***LowAlarm*** cause: The ***TMP*** has fallen below the ***LowAlarm*** limitPressure limits are set by ***Alarm_TMP*** .	

Alarm_Flux

Instruction name***Alarm_Flux***	Group***System:Settings:Alarms Method/Manual:Alarms&Monitors***
Parameter 1 name***Mode*** Parameter 2 name***High Alarm*** Parameter 3 name***Low Alarm***	Position name (default underlined)Disabled, Enabled Setpoint (default underlined)120 000.0 LMH (0.0-120 000.0) Setpoint (default underlined)0.0 LMH (0.0-120 000.0)
Instruction Help:Sets the alarm limits for the permeate flux rate. When reached the system is set to ***Pause***.	
Alarm Help text: ***HighAlarm*** cause: The permeate flux rate has exceeded the ***HighAlarm*** limit***LowAlarm*** cause: The permeate flux rate has fallen below the ***LowAlarm*** limitPressure limits are set by ***Alarm_Flux*** .	

Alarm_pNFF

Instruction name***Alarm_pNFF***	Group***System:Settings:Alarms Method/Manual:Alarms&Monitors***
Parameter 1 name***Mode*** Parameter 2 name***High Alarm*** Parameter 3 name***Low Alarm***	Position name (default underlined)Disabled, Enabled Setpoint (default underlined)5.20 bar (0.00-5.20) Setpoint (default underlined)0.00 bar (0.00-5.20)
Instruction Help:Sets the alarm limits for ***pNFF*** (feed pressure - permeate pressure). When reached the system is set to ***Pause***.	
Alarm Help: ***HighAlarm*** cause: The ***pNFF*** has exceeded the ***HighAlarm*** limit***LowAlarm*** cause: The ***pNFF*** has fallen below the ***LowAlarm*** limitPressure limits are set by ***Alarm_pNFF*** .	

Alarm_Shear

Instruction name***Alarm_Shear***	Group***System:Settings:Alarms Method/Manual:Alarms&Monitors***
Parameter 1 name***Mode*** Parameter 2 name***High Alarm*** Parameter 3 name***Low Alarm***	Position name (default underlined)Disabled, Enabled Setpoint (default underlined)20 000 s^{-1} (0-20 000) Setpoint (default underlined)0 s^{-1} (0-20 000)
Instruction Help:Sets the alarm limits for shear rate. When reached the system is set to ***Pause***.	
Alarm Help: ***HighAlarm*** cause: The shear rate has exceeded the ***HighAlarm*** limit***LowAlarm*** cause: The shear rate has fallen below the ***LowAlarm*** limitPressure limits are set by ***Alarm_pNFF*** .	

Alarm_Valves

Instruction name***Alarm_Valves***	Group***System:Settings:Alarms Method/Manual:Alarms&Monitors***
Parameter name***T_VB1*** ***T_VB2*** ***R_VB*** ***P_VB***	Position name (default underlined)Disabled, Enabled Disabled, Enabled Disabled, Enabled Disabled, Enabled
Instruction Help text:Enables/disables the transfer, retentate, and permeate valve alarms..	
Alarm Help:If a valve is not responding, an alarm is raised and the system is set to ***Pause***. Wait at least 5 seconds, then press the ***Continue*** button. If the alarm persists, try to restart the system. If the alarm still persists, check if the valve is damaged. If the valve is damaged, contact your GE service representative.	

Alarm_FlowPath

Instruction name***Alarm_FlowPath***	Group***System:Settings:Alarms Method/Manual:Alarms&Monitors***
Parameter 1 name***Mode***	Position name (default underlined)Enabled, Disabled
Instruction Help:Enables/Disables the transfer and permeate flow path alarms. The ***Alarm_FlowPath*** checks for closed flow paths (all transfer inlets closed, all permeate outlets closed) when transfer or permeate flow is > 0. An alarm will be raised and the system is set to ***Pause***.	
Alarm 1 Help: ***TrfFlow*** > 0 and inlets are closed ***Alarm 2 Help***: ***PermFlow*** > 0 and outlets are closed	

Alarm_Airsensor

Instruction name***Alarm_Airsensor***	Group***System:Settings:Alarms Method/Manual:Alarms&Monitors***
Parameter 1 name***Mode***	Position name (default underlined)Disabled, Enabled
Instruction Help:Sets the alarm for air sensor. An alarm will set the system to ***Pause*** if air is detected in air sensor flow cell.	

WatchPar_UV

Instruction name***WatchPar_UV***	Group***System:Settings:Alarms Method/Manual:Alarms&Monitors***
Parameter 1 name***Delta_Peak*** Parameter 2 name***Delta_Base***	Setpoint (default underlined)0.00 mAU (0.00-6000.00) 0.00 mAU (0.00-6000.00)

Instruction Help:Watch on UV signal. The ***DeltaPeak*** setting affects only the ***Less than or valley*** and ***Peak max*** conditions when using the ***Watch*** instruction (see the *UNICORN User Manual* for more information). As a general guideline, set the value to 2-3 times the noise level and 5-10% of the smallest expected peak height. ***Delta_Base*** setting affects only the ***Stable signal*** condition. For this condition to be activated, the signal may not vary by more than the ***Delta_Base*** value up or down over the time interval specified in the ***Stable signal*** condition in the ***Watch*** instruction.

WatchPar_Cond

Instruction name***WatchPar_Cond***	Group***System:Settings:Alarms Method/Manual:Alarms&Monitors***
Parameter 1 name***Delta_Peak*** Parameter 2 name***Delta_Base***	Setpoint (default underlined)<u>0.00</u> mS/cm (0.00-999.90) <u>0.00</u> mS/cm (0.00-999.90)
Instruction Help:Watch on conductivity signal. The ***Delta_Peak*** setting affects only the ***Less than or valley*** and ***Peak max*** conditions when using the ***Watch*** instruction (see the *UNICORN User Manual* for more information). As a general guideline, set the value to 2-3 times the noise level and 5-10% of the smallest expected peak height. ***Delta_Base*** setting affects only the ***Stable signal*** condition. For this condition to be activated, the signal may not vary by more than the ***Delta_Base*** value up or down over the time interval specified in the ***Stable signal*** condition in the ***Watch*** instruction.	

WatchPar_FeedPress

Instruction name***WatchPar_FeedPress***	Group***System:Settings:Alarms Method/Manual:Alarms&Monitors***
Parameter 1 name***Delta_Peak*** Parameter 2 name***Delta_Base***	Setpoint (default underlined)<u>0.00</u> bar (0.00-5.20) <u>0.00</u> bar (0.00-5.20)

Instruction Help:Watch on at pressure reported at the filter feed port. The ***Delta_Peak*** setting affects only the ***Less than or valley*** and ***Peak max*** conditions when using the ***Watch*** instruction (see the *UNICORN User Manual* for more information). As a general guideline, set the value to 2-3 times the noise level and 5-10% of the smallest expected peak height. ***Delta_Base*** setting affects only the ***Stable signal*** condition. For this condition to be activated, the signal may not vary by more than the ***Delta_Base*** value up or down over the time interval specified in the ***Stable signal*** condition in the ***Watch*** instruction.

WatchPar_RetenPress

Instruction name***WatchPar_RetenPress***	Group***System:Settings:Alarms Method/Manual:Alarms&Monitors***
Parameter 1 name***Delta_Peak*** Parameter 2 name***Delta_Base***	Setpoint (default underlined)<u>0.00</u> bar (0.00-5.20) <u>0.00</u> bar (0.00-5.20)
Instruction Help text:Watch on the pressure reported at the filter feed port. The ***Delta_Peak*** setting affects only the***Less than or valley*** and ***Peak max*** conditions when using the ***Watch*** instruction (see the *UNICORN User Manual* for more information). As a general guideline, set the value to 2-3 times the noise level and 5-10% of the smallest expected peak height. ***Delta_Base*** setting affects only the ***Stable signal*** condition. For this condition to be activated, the signal may not vary by more than the ***Delta_Base*** value up or down over the time interval specified in the ***Stable signal*** condition in the ***Watch*** instruction.	

WatchPar_PermPress

Instruction name***WatchPar_PermPress***	Group***System:Settings:Alarms Method/Manual:Alarms&Monitors***
Parameter 1 name***Delta_Peak*** Parameter 2 name***Delta_Base***	Setpoint (default underlined)<u>0.00</u> bar (0.00-5.20) <u>0.00</u> bar (0.00-5.20)

Instruction Help:Watch on the pressure reported at the filter permeate port. The ***Delta_Peak*** setting affects only the ***Less than or valley*** and ***Peak max*** conditions when using the ***Watch*** instruction (see the *UNICORN User Manual* for more information). As a general guideline, set the value to 2-3 times the noise level and 5-10% of the smallest expected peak height. ***Delta_Base*** setting affects only the ***Stable signal*** condition. For this condition to be activated, the signal may not vary by more than the ***Delta_Base*** value up or down over the time interval specified in the ***Stable signal*** condition in the ***Watch*** instruction.

WatchPar_FeedFlow

Instruction name***WatchPar_Feed-Flow***	Group***System:Settings:Alarms Method/Manual:Alarms&Monitors***
Parameter 1 name***Delta_Peak*** Parameter 2 name***Delta_Base***	Setpoint (default underlined)<u>0.0</u> mL/min (0.0-600.0) <u>0.0</u> mL/min (0.0-600.0)
Instruction Help:Watch on the feed flow rate. The ***Delta_Peak*** setting affects only the ***Less than or valley*** and ***Peak max*** conditions when using the ***Watch*** instruction (see the *UNICORN User Manual* for more information). As a general guideline, set the value to 2-3 times the noise level and 5-10% of the smallest expected peak height. ***Delta_Base*** setting affects only the ***Stable signal*** condition. For this condition to be activated, the signal may not vary by more than the ***Delta_Base*** value up or down over the time interval specified in the ***Stable signal*** condition in the ***Watch*** instruction.	

WatchPar_RetFlow

Instruction name***WatchPar_RetFlow***	Group***System:Settings:Alarms Method/Manual:Alarms&Monitors***
Parameter 1 name***DeltaPeak*** Parameter 2 name***Delta_Base***	Setpoint (default underlined)<u>0.0</u> mL/min (0.0-600.0) <u>0.0</u> mL/min(0.0-600.0)

Instruction Help:Watch on the retentate flow rate. The ***Delta_Peak*** setting affects only the ***Less than or valley*** and ***Peak max*** conditions when using the ***Watch*** instruction (see the *UNICORN User Manual* for more information). As a general guideline, set the value to 2-3 times the noise level and 5-10% of the smallest expected peak height. ***Delta_Base*** setting affects only the ***Stable signal*** condition. For this condition to be activated, the signal may not vary by more than the ***Delta_Base*** value up or down over the time interval specified in the ***Stable signal*** condition in the ***Watch*** instruction.

WatchPar_PermFlow

Instruction name***WatchPar_PermFlow***	Group***System:Settings:Alarms Method/Manual:Alarms&Monitors***
Parameter 1 name***Delta_Peak*** Parameter 2 name***Delta_Base***	Setpoint (default underlined)<u>0.0</u> mL/min (0.0-200.0) <u>0.0</u> mL/min (0.0-200.0)
Instruction Help:Watch on the permeate flow rate. The ***Delta_Peak*** setting affects only the ***Less than or valley*** and ***Peak max*** conditions when using the ***Watch*** instruction (see the *UNICORN User Manual* for more information). As a general guideline, set the value to 2-3 times the noise level and 5-10% of the smallest expected peak height. ***Delta_Base*** setting affects only the ***Stable signal*** condition. For this condition to be activated, the signal may not vary by more than the ***Delta_Base*** value up or down over the time interval specified in the ***Stable signal*** condition in the ***Watch*** instruction.	

WatchPar_TrfFlow

Instruction name***WatchPar_TrfFlow***	Group***System:Settings:Alarms Method/Manual:Alarms&Monitors***
Parameter 1 name***Delta_Peak*** Parameter 2 name***Delta_Base***	Setpoint (default underlined)<u>0.0</u> mL/min (0.0-200.0) <u>0.0</u> mL/min (0.0-200.0)

Instruction Help:Watch on the transfer flow rate. The ***Delta_Peak*** setting affects only the ***Less than or valley*** and ***Peak max*** conditions when using the ***Watch*** instruction (see the *UNICORN User Manual* for more information). As a general guideline, set the value to 2-3 times the noise level and 5-10% of the smallest expected peak height. ***Delta_Base*** setting affects only the ***Stable signal*** condition. For this condition to be activated, the signal may not vary by more than the ***Delta_Base*** value up or down over the time interval specified in the ***Stable signal*** condition in the ***Watch*** instruction.

WatchPar_RetVol

Instruction name***WatchPar_RetVol***	Group***System:Settings:Alarms Method/Manual:Alarms&Monitors***
Parameter 1 name***Delta_Peak*** Parameter 2 name***Delta_Base***	Setpoint (default underlined)<u>0.00</u> mL/min (0.00-80 000.00) <u>0.00</u> mL/min (0.00-80 000.00)
Instruction Help:Watch on the total retentate volume. The ***Delta_Peak*** setting affects only the ***Less than or valley*** and ***Peak max*** conditions when using the ***Watch*** instruction (see the *UNICORN User Manual* for more information). As a general guideline, set the value to 2-3 times the noise level and 5-10% of the smallest expected peak height. ***Delta_Base*** setting affects only the ***Stable signal*** condition. For this condition to be activated, the signal may not vary by more than the ***Delta_Base*** value up or down over the time interval specified in the ***Stable signal*** condition in the ***Watch*** instruction.	

WatchPar_ResVol

Instruction name***WatchPar_ResVol***	Group***System:Settings:Alarms Method/Manual:Alarms&Monitors***
Parameter 1 name***Delta_Peak*** Parameter 2 name***Delta_Base***	Setpoint (default underlined)<u>0.00</u> mL (0.00-80 000.00) <u>0.00</u> mL (0.00-80 000.00)

Instruction Help:Watch on the reservoir volume. The ***Delta_Peak*** setting affects only the ***Less than or valley*** and ***Peak max*** conditions when using the ***Watch*** instruction (see the *UNICORN User Manual* for more information). As a general guideline, set the value to 2-3 times the noise level and 5-10% of the smallest expected peak height. ***Delta_Base*** setting affects only the ***Stable signal*** condition. For this condition to be activated, the signal may not vary by more than the ***Delta_Base*** value up or down over the time interval specified in the ***Stable signal*** condition in the ***Watch*** instruction.

WatchPar_PermVol

Instruction name***WatchPar_PermVol***	Group***System:Settings:Alarms Method/Manual:Alarms&Monitors***
Parameter 1 name***Delta_Peak*** Parameter 2 name***Delta_Base***	Setpoint (default underlined)<u>0.00</u> mL (0.00-80 000.00) <u>0.00</u> mL (0.00-80 000.00)
Instruction Help:Watch on permeate volume. The ***Delta_Peak*** setting affects only the ***Less than or valley*** and ***Peak max*** conditions when using the ***Watch*** instruction (see the *UNICORN User Manual* for more information). As a general guideline, set the value to 2-3 times the noise level and 5-10% of the smallest expected peak height. ***Delta_Base*** setting affects only the ***Stable signal*** condition. For this condition to be activated, the signal may not vary by more than the ***Delta_Base*** value up or down over the time interval specified in the ***Stable signal*** condition in the ***Watch*** instruction.	

WatchPar_TransVol

Instruction name***WatchPar_TransVol***	Group***System:Settings:Alarms Method/Manual:Alarms&Monitors***
Parameter 1 name***Delta_Peak*** Parameter 2 name***Delta_Base***	Setpoint (default underlined)<u>0.00</u> mL (0.00-80 000.00) <u>0.00</u> mL (0.00-80 000.00)

Instruction Help:Watch on the transfer volume. The ***Delta_Peak*** setting affects only the ***Less than or valley*** and ***Peak max*** conditions when using the ***Watch*** instruction (see the *UNICORN User Manual* for more information). As a general guideline, set the value to 2-3 times the noise level and 5-10% of the smallest expected peak height. ***Delta_Base*** setting affects only the ***Stable signal*** condition. For this condition to be activated, the signal may not vary by more than the ***Delta_Base*** value up or down over the time interval specified in the ***Stable signal*** condition in the ***Watch*** instruction.

WatchPar_DeltaP

Instruction name***WatchPar_DeltaP***	Group***System:Settings:Alarms Method/Manual:Alarms&Monitors***
Parameter 1 name***Delta_Peak*** Parameter 2 name***Delta_Base***	Setpoint (default underlined)<u>0.00</u> bar (0.00-5.20) <u>0.00</u> bar (0.00-5.20)
Instruction Help:Watch on the feed DeltaP value. The ***Delta_Peak*** setting affects only the ***Less than or valley*** and ***Peak max*** conditions when using the ***Watch*** instruction (see the *UNICORN User Manual* for more information). As a general guideline, set the value to 2-3 times the noise level and 5-10% of the smallest expected peak height. ***Delta_Base*** setting affects only the ***Stable signal*** condition. For this condition to be activated, the signal may not vary by more than the ***Delta_Base*** value up or down over the time interval specified in the ***Stable signal*** condition in the ***Watch*** instruction.	

WatchPar_TMP

Instruction name***WatchPar_TMP***	Group***System:Settings:Alarms Method/Manual:Alarms&Monitors***
Parameter 1 name***Delta_Peak*** Parameter 2 name***Delta_Base***	Setpoint (default underlined)<u>0.00</u> bar (0.00-5.20) <u>0.00</u> bar (0.00-5.20)

Instruction Help:Watch on the transmembrane pressure. The ***Delta_Peak*** setting affects only the ***Less than or valley*** and ***Peak max*** conditions when using the ***Watch*** instruction (see the *UNICORN User Manual* for more information). As a general guideline, set the value to 2-3 times the noise level and 5-10% of the smallest expected peak height. ***Delta_Base*** setting affects only the ***Stable signal*** condition. For this condition to be activated, the signal may not vary by more than the ***Delta_Base*** value up or down over the time interval specified in the ***Stable signal*** condition in the ***Watch*** instruction.

WatchPar_Flux

Instruction name***WatchPar_Flux***	Group***System:Settings:Alarms Method/Manual:Alarms&Monitors***
Parameter 1 name***Delta_Peak*** Parameter 2 name***Delta_Base***	Setpoint (default underlined)<u>0.0</u> LMH (0.0-12 000.0) <u>0.0</u> LMH (0.0-12 000.0)
Instruction Help:Watch on the permeate flux rate reported by the permeate pump. The ***Delta_Peak*** setting affects only the ***Less than or valley*** and ***Peak max*** conditions when using the ***Watch*** instruction (see the *UNICORN User Manual* for more information). As a general guideline, set the value to 2-3 times the noise level and 5-10% of the smallest expected peak height. ***Delta_Base*** setting affects only the ***Stable signal*** condition. For this condition to be activated, the signal may not vary by more than the ***Delta_Base*** value up or down over the time interval specified in the ***Stable signal*** condition in the ***Watch*** instruction.	

WatchPar_Shear

Instruction name***WatchPar_Shear***	Group***System:Settings:Alarms Method/Manual:Alarms&Monitors***
Parameter 1 name***Delta_Peak*** Parameter 2 name***Delta_Base***	Setpoint (default underlined)<u>0</u> s^{-1} (0-20 000) <u>0</u> s^{-1} (0-20 000)

Instruction Help:Watch on the shear rate reported by the feed pump. The ***Delta_Peak*** setting affects only the ***Less than or valley*** and ***Peak max*** conditions when using the ***Watch*** instruction (see the *UNICORN User Manual* for more information). As a general guideline, set the value to 2-3 times the noise level and 5-10% of the smallest expected peak height. ***Delta_Base*** setting affects only the ***Stable signal*** condition. For this condition to be activated, the signal may not vary by more than the ***Delta_Base*** value up or down over the time interval specified in the ***Stable signal*** condition in the ***Watch*** instruction.

WatchPar_pNFF

Instruction name***WatchPar_pNFF***	Group***System:Settings:Alarms Method/Manual:Alarms&Monitors***
Parameter 1 name***Delta_Peak*** Parameter 2 name***Delta_Base***	Setpoint (default underlined)<u>0.00</u> bar (0.00-5.20) <u>0.00</u> bar (0.00-5.20)
Instruction Help:Watch on the ***pNFF*** (feed pressure - permeate pressure). The ***Delta_Peak*** setting affects only the ***Less than or valley*** and ***Peak max*** conditions when using the ***Watch*** instruction (see the *UNICORN User Manual* for more information). As a general guideline, set the value to 2-3 times the noise level and 5-10% of the smallest expected peak height. ***Delta_Base*** setting affects only the ***Stable signal*** condition. For this condition to be activated, the signal may not vary by more than the ***Delta_Base*** value up or down over the time interval specified in the ***Stable signal*** condition in the ***Watch*** instruction.	

WatchPar_%_Flux_Drop

Instruction name***WatchPar_%_Flux_Drop***	Group***System:Settings:Alarms Method/Manual:Alarms&Monitors***
Parameter 1 name***Delta_Peak*** Parameter 2 name***Delta_Base***	Setpoint (default underlined)<u>0.0</u>% (0.0-100.0) <u>0.0</u>% (0.0-100.0)

Instruction Help:Watch on the % flux drop reported by the permeate pump. The ***Delta_Peak*** setting affects only the ***Less than or valley*** and ***Peak max*** conditions when using the ***Watch*** instruction (see the *UNICORN User Manual* for more information). As a general guideline, set the value to 2-3 times the noise level and 5-10% of the smallest expected peak height. ***Delta_Base*** setting affects only the ***Stable signal*** condition. For this condition to be activated, the signal may not vary by more than the ***Delta_Base*** value up or down over the time interval specified in the ***Stable signal*** condition in the ***Watch*** instruction.

WatchPar_ConFactor

Instruction name***WatchPar_ConFactor***	Group***System:Settings:Alarms Method/Manual:Alarms&Monitors***
Parameter 1 name***Delta_Peak*** Parameter 2 name***Delta_Base***	Setpoint (default underlined)<u>0.00</u> (0.00-50.00) <u>0.00</u> (0.00-50.00)
Instruction Help text:Watch on ***concentration factor***. The ***Delta_Peak*** setting affects only the ***Less than or valley*** and ***Peak max*** conditions when using the ***Watch*** instruction (see the *UNICORN User Manual* for more information). As a general guideline, set the value to 2-3 times the noise level and 5-10% of the smallest expected peak height. ***Delta_Base*** setting affects only the ***Stable signal*** condition. For this condition to be activated, the signal may not vary by more than the ***Delta_Base*** value up or down over the time interval specified in the ***Stable signal*** condition in the ***Watch*** instruction.	

WatchPar_DF_X_Fact

Instruction name***WatchPar_DF_X_Fact***	Group***System:Settings:Alarms Method/Manual:Alarms&Monitors***
Parameter 1 name***Delta_Peak*** Parameter 2 name***Delta_Base***	Setpoint (default underlined)<u>0.00</u> (0.00-50.00) <u>0.00</u> (0.00-50.00)

Instruction Help:Watch on the diafiltration factor. The ***Delta_Peak*** setting affects only the ***Less than or valley*** and ***Peak max*** conditions when using the ***Watch*** instruction (see the *UNICORN User Manual* for more information). As a general guideline, set the value to 2-3 times the noise level and 5-10% of the smallest expected peak height. ***Delta_Base*** setting affects only the ***Stable signal*** condition. For this condition to be activated, the signal may not vary by more than the ***Delta_Base*** value up or down over the time interval specified in the ***Stable signal*** condition in the ***Watch*** instruction.

14.6 Fraction collector instructions

FracParameters

Instruction name***FracParameters***	Group***System*** →***Settings*** →***Specials***
Parameter 1 name***DelayVol*** Parameter 2 name***TubeChange***	Setpoint (default underlined)0.000 mL (0.000-10.000) Tube, DropSync, WasteBetweenTubes
Instruction Help:Parameter settings for the fraction collector. The delay volume is defined as the volume from the UV cell to the end of the tubing for the fraction collector. Collection of the flow during tube change can be handled in different ways. ***Tube***: no synchronisation of collection. ***DropSync***: tube change synchronised to drop release (this should only be used at flow rates generating drops). ***WasteBetweenTubes***: the flow will be diverted to waste when moving between tubes.	

FracNumberingMode

Instruction name***FracNumbering-Mode***	Group***System*** →***Settings*** →***Specials***
Parameter name***Mode***	Setpoint (default underlined)Reset, Continue
Instruction Help:Determines whether fraction number is reset at the end of a method or not. ***Reset*** sets Tube No to 1 after method end. ***Continue*** continues numbering from the last tube in the previous method.	

14.7 Monitor UPC-980

UV Monitor

UVLampOff

Instruction name***UVLampOff***	Group***Method/Manual →Alarms&Monitors***
Instruction Help:Sets the **UV lamp** permanently to ***OFF***. To restart UV lamp, the system and computer must be re-booted.	

pH

pHTempComp

Instruction name***pHTempComp***	Group***System →Settings →Monitors***
Parameter namepHTempComp	Position name (default underlined)OFF, ON
Instruction Help:Sets the temperature compensation ***ON*** or ***OFF***. For more accurate measurements during temperature changes, the pH measurement can be temperature compensated. When using ***pHTempComp*** it is important that the temperature of the pH electrode is the same as that of the conductivity flow cell since that is where the temperature is measured.	

Cond

CondTempComp

Instruction name***CondTempComp***	Group***System →Settings →Monitors***
Parameter nameCompFactor	Position name (default underlined)2.0% (0.0-9.9)

Instruction Help:Relates conductivity to temperature. The compensation consists of a compensation factor, together with a reference temperature (***CondRefTemp***). All conductivity values will automatically be converted to the set reference temperature. The factor is expressed in percentage increase of conductivity per °C increase in temperature. If the ***CompFactor*** is unknown, a general approximate value of 2% can be set for many common salt buffers. 0% = off.

CondRefComp

Instruction name***ConRefTemp***	Group***System*** →***Settings*** →***Monitors***
Parameter nameRefTemp	Position name (default underlined)25.0°C (0.0-99.9)
Instruction Help:Sets the reference temperature to which the measured conductivity values will be converted. ***CondRefTemp*** is active when a factor of ***CondTempComp*** is selected.	

14.8 Watch instructions

It is possible to set ***Watch*** and ***Watch_Off*** instructions on the following signals:***UV, pH, Cond, Feed_Press, RetenPress, PermPress, FeedFlow, RetFlow, PermFlow, TrfFlow, RetVol, PermVol, TransVol, DeltaP, TMP, Flux, Shear, pNFF, ConcFactor, DF_X_Fact, FluxDrop, Airsensor, ZeroLevel, AuxIn1, AuxIn2, AuxIn3,*** and ***AuxIn4***.

It is possible to set a ***Hold_Until*** instruction on the following signals:***UV, pH, Cond, Feed_Press, RetenPress, PermPress, FeedFlow, RetFlow, PermFlow, TrfFlow, RetVol, PermVol, TransVol, DeltaP, TMP, Flux, Shear, pNFF, ConcFactor, DF_X_Fact, FluxDrop, Airsensor, ZeroLevel, AuxIn1, AuxIn2, AuxIn3,*** and ***AuxIn4***.

14.9 Calibration

ZeroLS

Monitor***ZeroLS***	FunctionZero calibration of level sensor
Text 1Sets the level sensor reading to zero. See *Help*	

UPC

pH

Monitor***pH***	FunctionCalibration of the pH electrode
Text 1Calibrate pH electrode for buffer 1. See *Help*. Calibrate pH electrode for buffer 2. See *Help*.	
Parameter 1 nameReference value 1 Parameter 2 nameReference value 2	Input (default underlined)<u>0.0000</u> pH (0-14) Input (default underlined)<u>0.0000</u> pH (0-14)
ResultCalibrated electrode slope ResultAsymmetry potential at pH 7	Value 10.0% Value 20.0 mV
Instruction Help The pH Monitor is calibrated using standard buffer solutions in a two point calibration. The difference between the buffers should be at least 1 pH unit. A new electrode typically has a slope of 95-102% and an asymmetry potential within ± 30mV. As a rule, when an electrode has an asymmetry potential outside of ± 60mV and a slope lower than 80%, and no improvements can be achieved by cleaning, it should be replaced.	

Cond_Calib

Monitor***Cond_Calib***	FunctionCalibration of the conductivity cell constant
Text 1Determination of cell constant for Cond cell. See *Help*.	

Parameter nameReference value 1	Input (default underlined)1.0000 mS/cm (1-999.9)
ResultCalibrated electrode slope	Value1/cm
Instruction Help Calibration of conductivity cell. Normally it is not necessary to adjust the cell constant as the flow cell is pre-calibrated on delivery. Set ***CondTempComp*** to 0 in ***System*** →***Settings*** prior calibration. The temperature sensor must be calibrated before adjusting the cell constant. Fill the flow cell with calibration solution of 1.00 M NaCl. Wait until the temperature is constant in the range 20-30 °C. Enter the theoretical conductivity value according to graph in the *Operating Instructions* .	

Cond_Cell

Monitor***Cond_Cell***	FunctionEnter a new cell constant
Text 1Add cell constant value of a new Cond cell. See *Help*.	
Parameter nameReference value	Input (default underlined)40.0 1/cm (0.1-300)
Instruction Help When replacing the current cell with a new conductivity cell, enter the new cell constant (shown on the packaging.) In case the packaging has been discarded, perform the ***Cond_Calib***.	

Cond_Temp

Monitor***Cond_Temp***	FunctionTemperature sensor
Text 1Calibrate the temperature sensor in the conductivity flow cell. See *Help*.	
Parameter nameReference value	Input (default underlined)0.000°C (-5-60)

Instruction Help
Calibration of the temperature sensor in the conductivity flow cell is only necessary if very high accuracy measurements are needed. Place the conductivity flow cell together with a precision thermometer inside a box or empty beaker to make sure that they are not exposed to drafts. Leave them for 15 min to let the temperature stabilize. Read the temperature on the thermometer and enter this as the reference value.

TrfPress

Monitor***TrfPress***	FunctionPressure calibration
Text 1Sets the pressure reading on the transfer pressure sensor to zero. See *Help*.	
Parameter nameReference value	Input (default underlined)<u>0.00</u> bar (-5.0-60.0)
Instruction Help	
Calibrate pressure offset according to step 1:Calibrate pressure offset for **Transfer pump**. Click the ***Start Calibration*** button. For details see *ÄKTA Instrument Handbook*	

15 System components

About this chapter

This chapter contains information on the component parts of the ÄKTAcrossflow.

In this chapter

15.1 Pump P-982 and P-984

Pump P-982 and P-984 are high performance laboratory pumps for use in applications where accurately controlled liquid flow is required. Twin reciprocating pump heads work in unison to deliver a smooth and pulse-free flow.

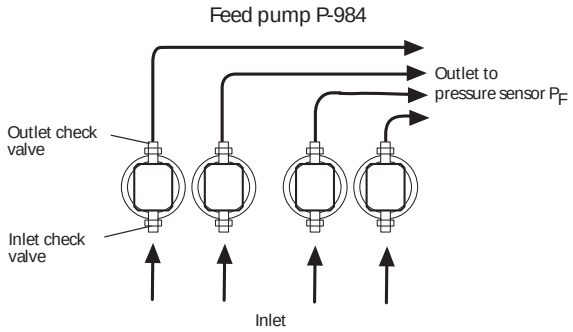
P-982 is used as the transfer pump (module A) and as the permeate pump (module B), P-984 is used as the feed pump (module A and B).

Pump	Pressure range	Flow rate range
P-982 (two pump heads)	0–52 kPa (5.2 bar, 75.4 psi)	0.1–200 mL/min
P-984 (four pump heads)	0–52 kPa (5.2 bar, 75.4 psi)	1–600 mL/min

Pump heads

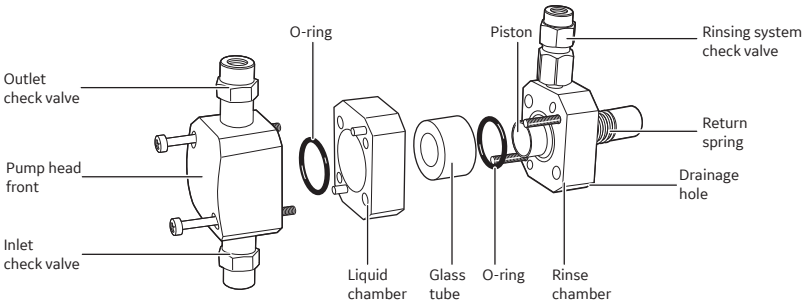
The individual pump heads are identical but are actuated in opposite phase to each other by individual stepper motors controlled by a microprocessor. This gives a continuous, low pulsation liquid delivery.

Each pump head is equipped with an inlet check valve and an outlet check valve for the liquid flow. In addition, each pump head has an outlet check valve for the rinsing system flow.



Liquid is drawn up into the pump head through a non-return inlet check valve by the action of the piston being withdrawn from the pump chamber.

On the delivery stroke of the piston, the inlet check valve is sealed by the pressure developed and liquid is forced out through a similar check valve at the outlet.

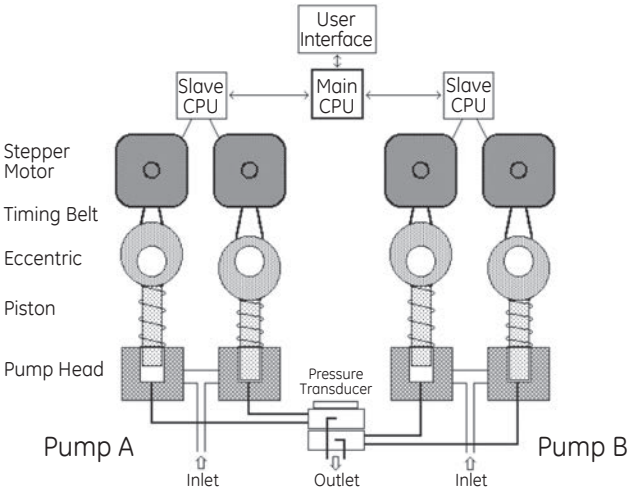


Leakage between the pump chamber and the drive mechanism is prevented by a piston. The piston is continuously lubricated by the presence of liquid. To prevent any deposition of salts from aqueous buffers on the pistons, the low pressure chamber behind the piston can be flushed continuously with a low flow of 20% ethanol.

The pump head is made of titanium alloy.

Pump principle

Each piston is driven by a simple robust cam (eccentric). These cams are driven by stepper motors via timing belts. The motor speed is varied to achieve linear movement and compensation for compressibility. This produces the particular motor sound. This system guarantees an accurate, low pulsation flow over the entire flow rate range, independent of the back pressure. When an increase in flow rate is programmed, the motor speed accelerates gradually, giving a soft start and building up speed to the flow rate required. When a decrease in flow rate is programmed, the motor speed slows rapidly to the lower flow rate.



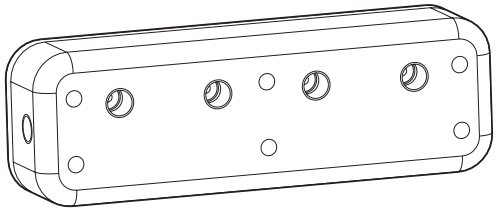
15.2 Valves

Membrane valves

Each valve block comprises three or four stepper-motor actuated membrane valves with open/close functionality. The valves are located in valve blocks to minimize holdup volumes and dead volumes.

A valve block consists of a connection block containing the ports and the membranes, and a mechanical housing containing the stepper motors, cams and actuating pistons. The membranes are made of EPDM.

The valve blocks have different numbers of inlet and outlet ports depending on their position in the flow path.



- Inlet valves T-VB-In: 1–4
- Inlet valves T-VB-In: 5–8
- Outlet valves P-VB-Out: recycle, 1, 2, 3 (pressure relief valve)

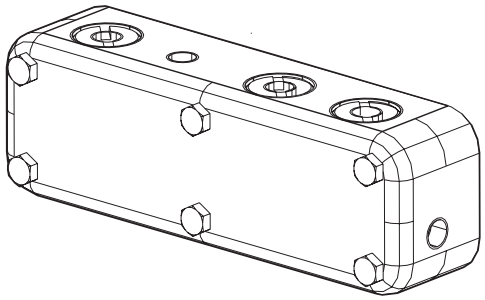
One of the outlet valves, P-VB-Out 3, is used as pressure relief valve with the opening pressure 7 bar (102 psi).

Rocker valve

The valve block comprises three stepper motor actuated diaphragm open/close valves. The diaphragm valve type comprises a membrane coated rocker.

The rocker closes against the flow through the inlet port with the closing force controlled by the stepper motor. This design results in linear control characteristics of the valve.

The valve block has different numbers of inlet and outlet ports depending on its position in the flow path.



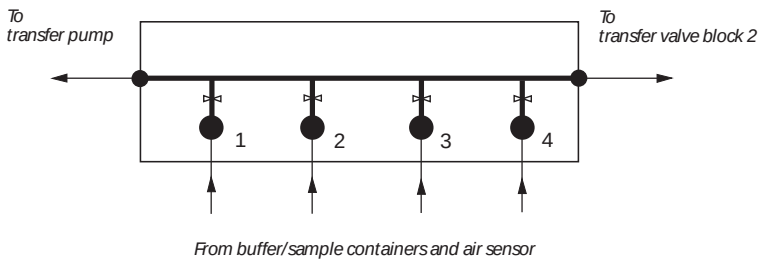
- Outlet valves R-VB-Out: 1 (pressure relief valve), 2, 3.

One of the outlet valves, R-VB-Out 1, is used as pressure relief valve with the opening pressure 7 bar (102 psi).

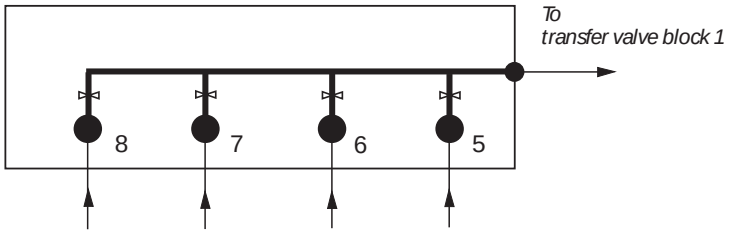
Valve block types

There are four different types of membrane valve blocks. The valve blocks have UNF 5/16" female ports. The following illustrations show the flow path in the valve blocks and where the valves are located. The valves are normally closed, with the exception of the open recirculation valve in the retentate valve block.

- Transfer valve block 1, T-VB-In 1, 2, 3 and 4

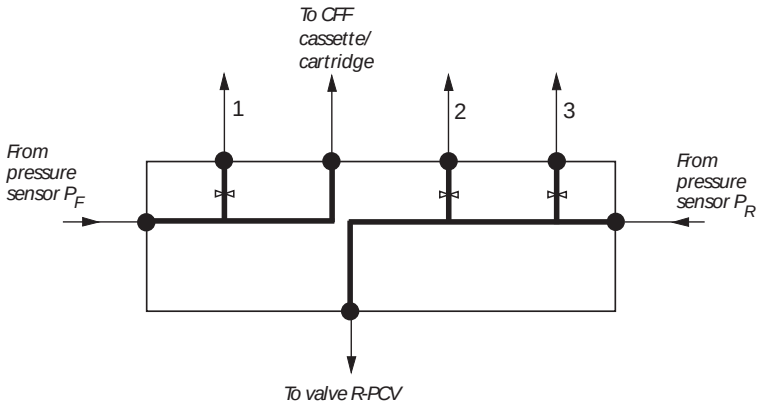


- Transfer valve block 2, T-VB-In 5, 6, 7 and 8

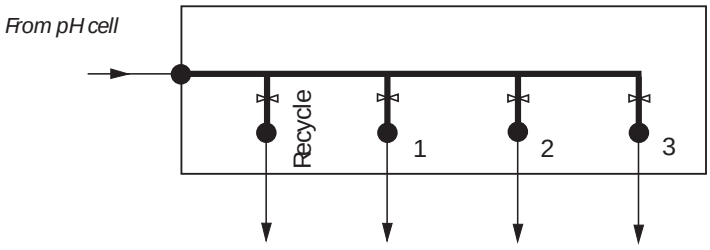


Note: *In transfer valve block 1 and 2, only one valve can be open at a time.*

- Retentate valve block, R-VB-Out 1, 2 and 3

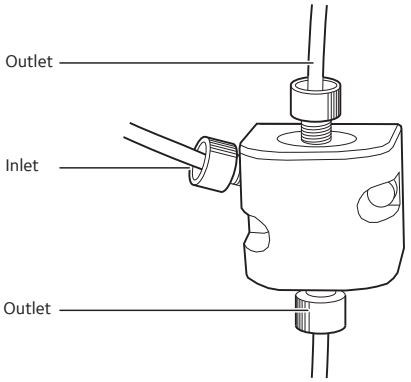


- Permeate valve block, P-VB-Out 1, 2, 3 and recycle



2-way transfer purge valve

The 2-way valve is a diaphragm type and comprises a membrane coated rocker. Actuated by a solenoid, the rocker blocks one of the two outlet ports in a flip-flop manner.



The inlet port is positioned at the side of the valve body, while the outlet ports are positioned at the top and bottom of the valve body.

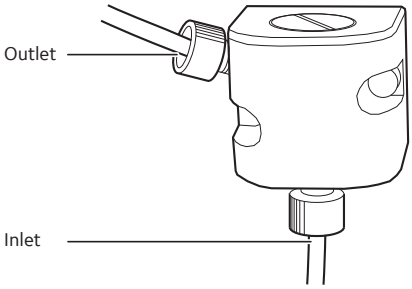
The transfer purge valve directs the liquid flow either from transfer line or permeate recycle towards the reservoir (default) or waste.

Pressure modulating valves R-PCV and P-PCV

The pressure control valves enable a throttling of the liquid flow in order to raise the pressure upstream the valve.

Mechanically, these valves are similar to the 2-way valve such that the throttling of the flow is achieved by the membrane coated rocker. However, compared to the 2-way switch valve, the pressure control valve has only one inlet and one outlet port.

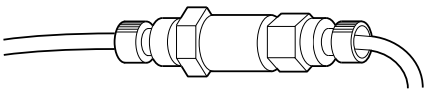
The rocker closes against the flow through the inlet port with the closing force controlled by the solenoid. This design results in linear control characteristics of the valve. Furthermore, the pressure upstream of the valve is maintained irrespective of changes in flow rate.



- Retentate control valve (R-PCV)The retentate control valve R-PCV is used to accurately control the retentate pressure over the pressure range 0.1 to 5.2 bar. Hereby, the transmembrane pressure (TMP) can be adjusted. In addition, the R-PCV can operate as open/close valve in product recovery and system cleaning procedures.
- Permeate control valve (P-PCV)The main task of the permeate control valve P-PCV is to modulate the pressure downstream of the permeate pump in order to guarantee accuracy in the permeate flow rate.For proper operation of the check valves, the pressure downstream of the pump must be greater than the pressure upstream the pump. Therefore, the P-PCV is controlled such that it always maintains a higher pressure downstream of the pump.

Flow restrictor in transfer line

A flow restrictor is positioned downstream of the transfer pump for proper operation of the check valves at the pump heads. The restrictor generates a minimum constant back pressure of 3 bar.



15.3 Reservoirs

The reservoir accommodates the liquid/sample to be processed. It provides a gentle, but efficient mixing of the process liquid with returning retentate and additional liquid added via the transfer line. Permeate may be recycled into the reservoir for achieving steady-state conditions during process development studies.

The reservoirs are equipped with a float to prevent vortex formation and foaming such that operation at lowest recirculation volume with high flow rate is facilitated.

There are two sizes of reservoirs:

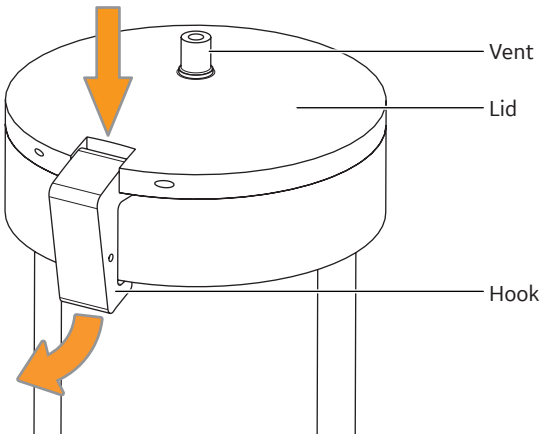
- 350 mL (375 mL without float)
- 1100 mL (1200 mL without float) (optional)

Each reservoir has connections for the liquid flow positioned at the reservoir bottom end plate. There is one outlet for delivering liquid to the feed pump via a manifold. The outlet is placed off-centre at the bottom of the reservoir to prevent vortex formation. The outlet is connected to a conduit/manifold that distributes the liquid to the four pump heads of the feed pump. The retentate return is positioned such that liquid is injected tangentially to the bottom surface.

The lid can be easily opened, for example for manual sampling of the retentate. It also has a connection for ventilation.

To open the lid:

Step	Action
	Move the lower part of the jointed hook outwards while pushing slightly the lid downwards.



The reservoir is mounted on a reservoir holder which comprises a motor unit for a magnetic stirrer-bar. The stirrer can be used with both reservoirs to improve mixing characteristics. Recommended dimensions for the stirrer are:

- 350 mL reservoir: length of stirrer 30 mm and o.d. 6 mm
- 1100 mL reservoir: length of stirrer 35 mm and o.d. 6 mm

The appropriate mixing rate is a function of application and retentate volume and can be adjusted by the control software. At low retentate volume, the stirrer and the float will be in contact such that the stirrer will rotate the float.

Under these conditions, a low mixing rate is selected as default by the control software. At higher retentate volume where the float is not in contact with the stirrer, the user can select a higher mixing rate. The following rates are recommended as maximum mixing rates that provide sufficient mixing for all conditions:

- 350 mL reservoir: 200 rpm
- 1100 mL reservoir: 300 rpm

As default, the UNICORN control software adjusts the mixing rate automatically depending on the actual retentate volume.

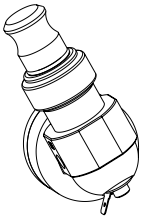
An air filter (vent filter) can be connected to the top of the reservoir.

The reservoir consists of the following material:

- glass tube: borosilicate
- bottom end plate, top flange and lid: polyetherimide
- sealing lid: thermoplastic elastomer
- float: polypropylene
- stirrer: polytetrafluoroethylene

15.4 pH electrode and cell holder

The pH electrode is optimized for continuous pH measurement in the permeate flow path. The electrode is of the sealed combination double junction type. It contains a sealed Ag/AgCl reference, which cannot be refilled, an internal electrolyte bridge of 4 M KCl saturated with Ag/AgCl, an outer electrolyte bridge of 1 M KNO_3, an annular ceramic reference junction, and a low profile pH membrane.



The pH electrode has a glass tip and the cell holder is made of titanium. The whole assembly is replaceable.

The pH electrode should be calibrated regularly. This procedure is described in *Section 3.8 Calibrate the pH electrode, on page 53*.

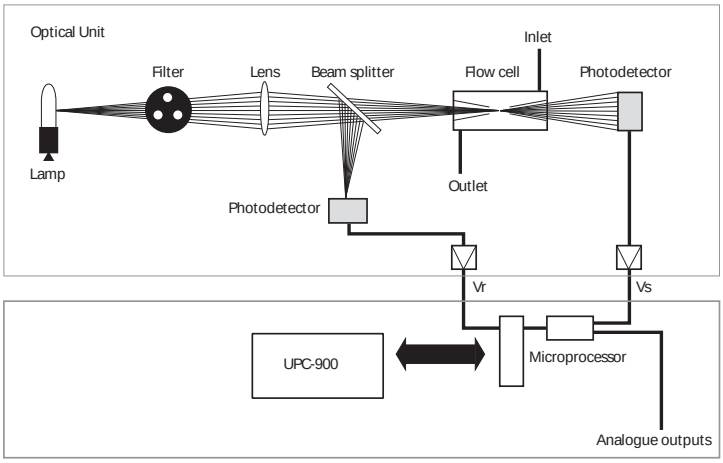
15.5 Monitor UPC-980 and UV cell

The UV cell is designed for continuous measurement of UV absorbance. The UV monitoring system provides high performance detection for the wavelength of 254 or 280 nm. Additional filters can be purchased to provide monitoring of the wavelengths 313, 365, 405, 436, and 546 nm. With the optional zinc lamp, the wavelength 214 nm can be monitored.

The UV cell housing is made of PEEK. Other wetted parts are made of glass and titanium.

UV optical unit

The UV optical unit houses the lamp (Zn or Hg), the wavelength filter and the UV flow cell. The light beam is directed through a flow-through cuvette to a photodetector. The photodetector current is fed to the signal processing circuitry in the module.



The reference signal comes from the same point in the lamp as the signal measuring the sample, thus assuring a stable baseline by eliminating the effects of variations in lamp intensity.

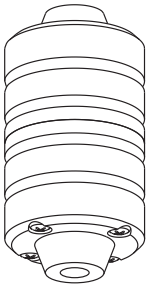
The Hg lamp emits light only at certain wavelengths. It does not emit light at 280 nm, so for this wavelength, the light is converted at a fluorescent surface before it passes the filter. On the lamp housing, there is a special exit for 280 nm light, which means that the lamp position needs to be changed when working with this wavelength.

For 214 nm wavelength, a Zn lamp is used. This lamp is larger than the Hg lamp and is therefore mounted in a larger lamp housing.

The lamp connectors are keyed to inform the monitor software of which lamp type is connected.

15.6 Conductivity cell

The cell has two cylindrical titanium electrodes positioned in the flow path of the cell. An alternating voltage is applied between the electrodes and the resulting current is measured and used to calculate the conductivity of the buffer. The system controls the AC frequency and increases it with increasing conductivity between 50 Hz and 50 kHz, giving maximum linearity and true conductivity values.



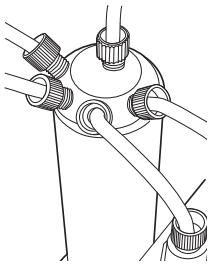
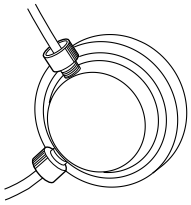
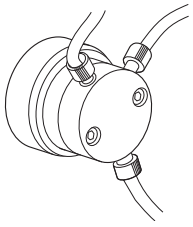
The conductivity is automatically calculated by multiplying the measured conductance by the cell constant of the cell. The cell constant is precalibrated on delivery but can be measured with a separate calibration procedure. This procedure is described in *Section 3.9 Calibrate the conductivity cell, on page 55*.

One of the electrodes has a small temperature sensor for measuring the temperature of the buffer in the cell. Temperature variations influence the conductivity and in some applications, when very precise conductivity values are required, it is possible to program a temperature compensation factor that recalculates the conductivity to a set reference temperature.

15.7 Sensors

The ÄKTAcrossflow system is equipped with four pressure sensors, one level sensor, and one air sensor.

Pressure sensors

Pressure sensor P_F	Located close to the CFF cassette/cartridge in the feed line to measure the feed pressure.	
Pressure sensor P_R	Located close to the CFF cassette/cartridge in retentate line to measure the retentate pressure.	
Pressure sensor P_P	Located close to the CFF cassette/cartridge in the permeate line to measure the permeate pressure.	
Pressure sensor P_T	Located upstream of the reservoir, the transfer pressure sensor is used to maintain safe operation by measuring the pressure in the reservoir. The liquid chamber in the P_T sensor housing is equipped with a thin titanium membrane. A strain gauge is attached to the rear side of the membrane. When the liquid pressure increases, the titanium membrane bulges, which is detected by the strain gauge.	

The pressure is shown on the computer display. To protect the system, a maximum and minimum pressure limit can be set in UNICORN for pressure sensors P_F, P_R and P_P.

The pressure sensors have a pressure range of 0–10 bar (100 kPa, 145 psi). The pressure sensor housing is made of PEEK. Other wetted parts are made of titanium and stainless steel.

Reservoir level sensor

The reservoir level sensor is located in the reservoir bottom end plate. The level sensor also has the function of low volume alarm for the reservoir.

The signal of the level sensor is used to maintain a consistent volume in the reservoir during constant volume operations (fed batch concentration and diafiltration). Furthermore, the level sensor facilitates efficient product removal procedures at the end of the filtration process in case that any entrainment of air in the recirculation line is not desirable.

The level sensor has a pressure range of 0–100 mbar (10 kPa, 1.45 psi).

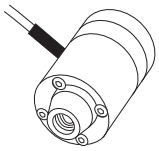
A temperature sensor is integrated with the reservoir level sensor, and allows for continuous measurement of the liquid feed to the CFF cassette/cartridge.

CAUTION

The reservoir level sensor is highly sensitive. Do not insert any objects into the cavity in the bottom end plate of the reservoir since this may damage the level sensor.

Air sensor 925

The sample air sensor is a high precision monitor designed for continuous monitoring of air bubbles in the flow path for the sample inlet. The air sensor is made of PEEK.



The air sensor makes sure that the maximum volume of external feed can be transferred into the system without any risk for introducing air into the transfer line. When air is detected, the system is either paused, or performs an action that is set in the method.

Avoiding air in the transfer line is necessary to provide the high volume accuracy of the transfer pump and thereby the accuracy of the retentate volume content.

Appendix A
Membrane and cartridge selection

Cell processing

When selecting membranes for clarification, smaller pore size filters resist fouling more than filters with larger pore sizes such as 0.45 or 0.65 µm. A general guideline is to select the smallest pore size ratings that is at least 10× larger than the size of the target protein in it largest state or longest dimension. When working with lysates, which can contain a wide range of particle sizes and many types of proteins and sticky cell components, choosing a small pore size can help prevent fouling of the membrane pores.

When selecting cartridges, the presence of particles in the feed stream requires the selection of short path length cassettes (30 to 60 cm) with large lumen diameters (0.75 to 1.0 mm). See *Section B Tubing specifications, on page 334*.

Concentration and diafiltration

A membrane with a molecular weight cutoff that is too large will allow the molecule of interest to pass through and significantly reduce yields. Conversely a membrane with too small an molecular weight cutoff will reduce flux and lead to slower processing times, oversized systems, increased capital cost, and plant space requirements, working volume and hold-up volume. For typical membrane-based crossflow applications, the membrane pore size selection is based on the size of the target molecule.

The general guideline for selecting a membrane for product concentration is to start with a molecular weight cutoff that is 3 to 5 times smaller than the target molecule. For example, a 50 kD or 30 kD membrane would be a suitable choice to retain IgG (160 kD), and a 30 kD or 10 kD membrane would be a suitable choice for albumin (66 kD).

Appendix B
Tubing specifications

The ÄKTAcrossflow is supplied with two tubing kits for the recirculation line to accommodate applications and filters run at low and high flow rates as follows:

- Tubing with an inner diameter of 1.7 mm (Small) for low flow rate applications (typically < 80 mL/min feed flow rate)
- Tubing with an inner diameter of 2.9 mm (Large) for high flow rate applications (typically > 80 mL/min feed flow rate)

The system holdup volume and thus the working volume is minimized when using tubing with the small diameter.

Note: *The working volume is reservoir volume + system holdup volume + cassette/cartridge holdup volume.*

Table 16.1: Recommended combinations of filters and tubing diameters

Filter cassette/cartridge	Application	Recommended tubing
Flat sheet, 100 cm^2	UF/DF	S = i.d. 1.7 mm
Flat sheet > 100 cm^2	UF/DF	L = i.d. 2.9 mm[1]
Hollow fiber, Start AXH	UF/DF	S = i.d. 1.7 mm
Hollow fiber, Start AXM	UF/DF	S = i.d. 1.7 mm[2]
Hollow fiber, Start AXM	MF	L = i.d. 2.9 mm

[1] Small i.d. tubing might be applicable depending on application.

[2] Large i.d. tubing may be applicable for high flow/high viscosity.

Table 16.2: Recommended tubing for Start AXM hollow fiber membrane cartridges

Tubing	length (mm)	o.d. (mm)	i.d. (mm)	Volume (mL)	Material	Location from	Location to
F1S	300	3	1.7	0.68	PVDF	Valve block R-VB	Cartridge, feed inlet

Tubing	length (mm)	o.d. (mm)	i.d. (mm)	Volume (mL)	Material	Location from	Location to
F1L	300	4.76 (3/16")	2.9	1.98	ETFE		
R1S	200	3	1.7	0.45	PVDF	Cartridge, retentate outlet	Sensor $P_{R,}$ inlet
R1L	200	4.76 (3/16")	2.9	1.32	ETFE		
P1S	155	3	1.7	0.35	PVDF	Cartridge, permeate outlet	Sensor $P_{P,}$ inlet
P1L	150	4.76 (3/16")	2.9	0.99	ETFE		

Table 16.3: Recommended tubing for Start AXH hollow fiber membrane cartridges

Tubing	length (mm)	o.d. (mm)	i.d. (mm)	Volume (mL)	Material	Location from	Location to
F1S	200	3	1.7	0.45	PVDF	Valve block R-VB	Cartridge, feed inlet
R1S	200	3	1.7	0.45	PVDF	Cartridge, retentate outlet	Sensor $P_{R,}$ inlet
P1S	155	3	1.7	0.35	PVDF	Cartridge, permeate outlet	Sensor $P_{P,}$ inlet

Retentate volume setup

The retentate volume is the sum of the retentate holdup volume and the liquid volume in the reservoir:

RetVol = RetentateHoldupVolume + ResVol

The reservoir volume in the ÄKTAcrossflow system is based on pumped volumes reported by the pumps. In order to calculate the correct retentate volume, user-defined input on the retentate holdup volume is required. The retentate holdup volume is the sum of system holdup volume (in components and tubing) and the retentate volume in the filter:

RetentateHoldupVolume = system holdup volume + filter volume

The system holdup volume depends on the tubing configuration.

System holdup volume and recommended minimum working volume

Recirculation tubing kit	Feed tubing	Retentate tubing	Permeate tubing	System holdup volume (mL) [1]	Minimum working volume (mL) [2]	
					350 mL reservoir	1100 mL reservoir
Small i.d. (1.7 mm)	F1S 200	R1S 200	P1S 155	18.2	24 (22.2)	40 (26.2)
Small i.d. (1.7 mm)	F1S 300	R1S 200	P1S 155	18.4	24 (22.4)	45 (26.4)
	F1S 200	R1S 300				
Large i.d. (2.9 mm)	F1L 200	R1L 300	P1L 150	25.8	32 (29.8)	50 (33.8)
	F1L 300	R1L 200				

[1] System holdup volumes do not account for filter volume: ***RetentateHoldupVolume*** = system holdup volume + filter volume.

[2] Recommended minimum working volume accounts for accuracy in control and measurement of the retentate volume. The figures in brackets state typical values for the lowest possible working volume (excluding filter volume).

Hollow fiber cartridges with UNF fittings

Name	Cut-off	Inner diam-eter (mm)	Length (cm)	Num-ber of fibers	Nom-inal area (cm^2)	Feed flow rate 2000-16000 s^{-1}	Flux range low = 10 LMH	Flux range high = 80 LMH	30-200L /m^2 vol chal-lenge
Start AXH	3 kD	0.5	60	4	40	8.5-66 mL/min	0.7 mL/min	5.3 mL/min	0.12-0.80
Start AXH	10 kD	0.5	60	4	40	8.5-66 mL/min	0.7 mL/min	5.3 mL/min	0.12-0.80
Start AXH	30 kD	0.5	60	4	40	8.5-66 mL/min	0.7 mL/min	5.3 mL/min	0.12-0.80
Start AXH	100 kD	0.5	60	4	40	8.5-66 mL/min	0.7 mL/min	5.3 mL/min	0.12-0.80
Start AXH	300 kD	0.5	60	4	40	8.5-66 mL/min	0.7 mL/min	5.3 mL/min	0.12-0.80
Start AXH	500 kD	0.5	60	4	40	8.5-66 mL/min	0.7 mL/min	5.3 mL/min	0.12-0.80
Start AXH	750 kD	0.5	60	4	40	8.5-66 mL/min	0.7 mL/min	5.3 mL/min	0.13-0.84
Start AXM	500 kD	1.0	30	6	50	70-600 mL/min	0.8 mL/min	6.7 mL/min	0.15-1.0
Start AXM	750 kD	1.0	30	6	50	70-600 mL/min	0.8 mL/min	6.7 mL/min	0.15-1.0

Name	Cut-off	Inner diam-eter (mm)	Lengt h (cm)	Num-ber of fibers	Nom-inal area (cm^2)	Feed flow rate 2000-16000 s^{-1}	Flux range low = 10 LMH	Flux range high = 80 LMH	30-200L /m^2 vol chal-lenge
Start AXM	3 kD	0.5	30	12	50	25-200 mL/min	0.8 mL/min	6.7 mL/min	0.15-1.0
Start AXM	10 kD	0.5	30	12	50	25-200 mL/min	0.8 mL/min	6.7 mL/min	0.15-1.0
Start AXM	30 kD	0.5	30	12	50	25-200 mL/min	0.8 mL/min	6.7 mL/min	0.15-1.0
Start AXM	100 kD	0.5	30	12	50	25-200 mL/min	0.8 mL/min	6.7 mL/min	0.15-1.0
Start AXM	300 kD	0.5	30	12	50	25-200 mL/min	0.8 mL/min	6.7 mL/min	0.15-1.0
Start AXM	500 kD	0.5	30	12	50	25-200 mL/min	0.8 mL/min	6.7 mL/min	0.15-1.0
Start AXH	750 kD	0.5	30	12	50	25-200 mL/min	0.8 mL/min	6.7 mL/min	0.15-1.0
Start AXM	0.1 µm	1.0	30	6	50	70-600 mL/min	0.8 mL/min	6.7 mL/min	0.15-1.0

Name	Cut-off	Inner diam-eter (mm)	Length (cm)	Num-ber of fibers	Nom-inal area (cm^2)	Feed flow rate 2000-16000 s^{-1}	Flux range low = 10 LMH	Flux range high = 80 LMH	30-200L /m^2 vol chal-lenge
Start AXM	0.2 µm	1.0	30	6	50	70-600 mL/min	0.8 mL/min	6.7 mL/min	0.15-1.0
Start AXM	0.45 µm	1.0	30	6	50	70-600 mL/min	0.8 mL/min	6.7 mL/min	0.15-1.0
Start AXM	0.65 µm	0.75	30	8	50	40-320 mL/min	0.8 mL/min	6.7 mL/min	0.15-1.0

Appendix C
Technical specifications

Transfer pump and permeate pump P-982

Flow rate	0.1–200 mL/min
Increment	0.1 mL
Pressure	0–520 kPa (5.2 bar, 75.4 psi)
Flow rate accuracy	0.5% actual volume within range (2–200 mL/min, 3.0 - 5.0 bar)
Pulsation	Max. ± 10% at inlet side (with inlet flow 10 mL/min, 4 bar) Max. ± 20% at outlet side (with outlet flow 10 mL/min, 4 bar)
Flow rate reproducibility	rsd < 0.15% (0.1–200 mL/min, 3.0 - 5.0 bar)
Viscosity	0.8 - 5.0 cP
Internal volume	3050 µL including check valves

Feed pump P-984

Flow rate	0.1–600 mL/min
Increment	0.1 mL
Pressure	0–520 kPa (5.2 bar, 75.4 psi)
Flow rate accuracy	< ± 2% actual volume within range (2 - 600 mL/min, 2.0 - 5.2 bar)

Pulsation	Max. ± 10% at inlet side (with inlet flow 10 mL/min, 4 bar) Max. ± 20% at outlet side (with outlet flow 10 mL/min, 4 bar)
Flow rate reproducibility	rsd < 0.3% (2 - 600 mL/min, 2.0 - 5.2 bar)
Viscosity	0.8–5.0 cP
Internal volume	6100 µL including check valves
Liquid exchange between product side and rinsing system	< 4.5 ppm of pumped volume

UV measurement, Monitor UV-980

UV cell path length	2 mm
UV cell flow area	1.6 mm^2
UV cell total holdup volume	0.21 mL
Baseline adjust	Adjustable 0–100% of full scale
Static drift	± 100 × 10^{-6} AU/h at 254 nm
Autozero range	-0.2–2.0 AU
Absorbance range	0.01–5 AU

pH measurement, Monitor pH/C-980

pH range	0–14 (spec. valid between 2 and 12)

Accuracytemperature compensatednot temperature compensated	± 0.1 pH units within 4°C - 40°C ± 0.2 pH units within 15°C - 25°C ± 0.5 pH units within 4°C–15°C and 25°C–40°C
Response time	Max. 10 s (0–95% of step)
Long-term drift	Max. 0.1 pH units/10 h
Flow rate sensitivity	Max. 0.1 pH units within 0–100 mL/min
Max. pressure	0.5 MPa (5 bar, 72 psi)
Internal volume, pH cell holder	240 µL

Conductivity measurement, Monitor pH/C-980

Conductivity range	1 µS/cm to 250 mS/cm
Deviation from theoretical conductivity	Max. ± 2% of full scale calibrated range or ±10 µS/cm whichever is greater in the range 1 µS/cm to 250 mS/cm
Reproducibilityshort-termlong-term	Max. ± 1% or ± 5 µS/cm Max. ± 3% or ± 15 µS/cm
Noise	Max. ± 0.5% of full scale calibrated range
Response time	Max. 3 s (0–95% of step)
Flow rate sensitivity	± 1% within 0–400 mL/min
Max. pressure	2 MPa (20 bar, 290 psi)
Internal volume, conductivity cell	180 µL

Membrane valves

Max. pressure	520 kPa (5.2 bar, 75.4 psi)

Internal volume T-VB 1 and 2 P-VB	570 µL (closed) 570 µL (closed)
Valve principle	Stepper motor-controlled membrane

Rocker valve

Max. pressure	520 kPa (5.2 bar, 75.4 psi)
Internal volume R-VB, retentate side R-VB, feed side	540 µL (closed) 390 µL (closed)
Valve principle	Stepper motor-controlled membrane

Control valves R-PCV and P-PCV

Max. pressure	520 kPa (5.2 bar, 75.4 psi)
Internal volume R-VB, retentate side R-VB, feed side	540 µL, 520 µL (closed) 540 µL, 520 µL (closed)
Valve principle	Solenoid-actuated rocker

Flow restrictor (Transfer line)

Back pressure	Min. 3 bar
Internal volume	570 µL
Valve principle	Spring-loaded cone

Transfer purge valve

Max. pressure	520 kPa (5.2 bar, 75.4 psi)
Internal volume	600 µL, 580 µL (closed)
Valve principle	Solenoid-actuated rocker, membrane coated

Reservoirs

Max. volume Without float With float	375 mL, 1200 mL350 mL, 1100 mL
Mixing principle	Magnetic stirrer

Pressure sensors P_F, P_R, P_P

Pressure range	Up to 1 MPa (10 bar, 145 psi)
Pressure accuracy	± 0.01 bar
Internal volume:P_F P_R, P_P	565 µL 340 µL

Pressure sensor P_T

Pressure range	0–2.5 MPa (25 bar, 362 psi)
Pressure accuracy	< ± 2%
Internal volume	294 µL

Reservoir level sensor

Pressure range	0–100 mbar (10 kPa, 1.45 psi)

Reproducibility in empty reservoir detection	± 0.2 mL
Drift under constant retentate volume operation	± 0.1 mbar (10 Pa) over 4 hours, valid for temperature changes ≤ 1 °C/hour (for water, a hydrostatic pressure of 10 Pa corresponds to approx. 2.8 mL in the small reservoir, and 6.4 mL in the large reservoir)

Temperature sensor

Integrated with reservoir level sensor

Accuracy	± 1°C (valid for temperature difference < 5°C between liquid temperature and ambient temperature)

Air sensor 925

Max. pressure	2.5 MPa (25 bar, 362 psi)
Internal volume	190 µL

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