MiniKros® Sampler System

Spectrum Laboratories, Inc.
18617 Broadwick Street
Rancho Dominguez, CA 90220
Voice: (310) 885-4600 • (800) 634-3300
Fax: (310) 885-4666
e-mail: customerservice@spectrumlabs.com

Spectrum Product Instruction Booklet

400-02219-000 Rev01

SPECTRUM LABS.COM

1. MiniKros® Sampler System

Hollow Fiber Tangential Flow Filtration System for R&D Scale Volumes

Illustration 1.1 - MiniKros® Sampler System for processing volumes from 50 ml to 3 liters.

Specifications

Major Components:
- MP-2 Peristaltic Pump (1.6 LPM)
- KrosFlo® Pro Pressure/Flow Monitor
- Disposable Pressure Transducer
- 60 ml Process Reservoir
- 2 liter Feed Reservoir
- Disposable Flow Path (tubing and connections)

Process Volume:	50 ml – 3 liters
Filtrate Rate:	10 – 1,000 ml/min
Process Time:	30 min to 6 hrs

Table 1.1 - MiniKros® Sampler System Specifications

R & D Applications:

- Diafiltration of Diagnostic Particles
- Cell Washing and/or Harvesting
- Clarification of Cellular Debris
- Protein Purification and Concentration
- Purification and Concentration of Virus

Table 1.2 - MiniKros Sampler System R & D Applications

The MiniKros Sampler System is ideal for processing R&D scale sample volumes and for filtration characterization for larger volume applications. This system features the KrosFlo Pro Pressure/Flow Monitor for tracking process parameters. Used in conjunction with disposable MiniKros Sampler modules (120 – 615 cm^2), this system is designed for processing volumes ranging from 50 ml – 3 liters with permeate rates ranging from 10 ml/min to 1 L/min depending on the application and filter MWCO. All Spectrum hollow fiber filters and systems are engineered for direct process scale up.

MiniKros Sampler System Operation: Total processing in LESS THAN 2 HOURS

The peristaltic pump circulates the sample through the lumen of the filter hollow fibers. While clarified filtrate passes through the fiber walls, the retentate is returned to the process reservoir for further concentration or purification. At the same rate filtrate passes through, more sample or wash solution is pulled into circulation from the feed reservoir.

2. Contents of MiniKros® Sampler System

ACPU-111-E1N	110 VAC peristaltic pump with easyload pump head (or ACPU-222-E1N for 220 VAC)
ACMS-407-01N	pressure monitor w/digital readout, 110 VAC, w/transducer (or ACMS-408-0l N for 220 VAC)
ACTO-P06-O1N	60 ml reservoir with two hose barb fittings and one vent
ACMS-400-01N	Setup kit for MiniKros Sampler System

ACMS-400-01N Setup kit for MiniKros® Sampler System includes:

Description	Quantity	Description	Quantity
Size 16 Silicone Tubing	60 cm	Female Luer to 5 mm Hose Barb Adaptors, Polypropylene	three
Size 17 Silicone Tubing	90 cm	Female Luer Tees, Polypropylene	two
Luer Check Valve, Polycarbonate	one	Female Luer to Female Luer Connectors, Polypropylene	two
Male Luer Caps, Polypropylene	two	Inflation Bulb w/Vent Valve	one
Male Luer to 3 mm Hose Barb Adaptors, Polypropylene	five	Clear 12 mm Y Connectors, PVC	two
Size 1 Hose Clamps	two	Square 2 Liter Vessel with Filling/Venting Closure	one
Size 2 Hose Clamps	one	Metal Bar for holding Clips	one
Size 4 Hose Clamps	two	Vessel Submersion Tubes, Polypropylene:	two
Hose Shut-Off Clamps	two		
Clips for holding Module	two		
Thin Wall Silicone Tubing	15 cm		

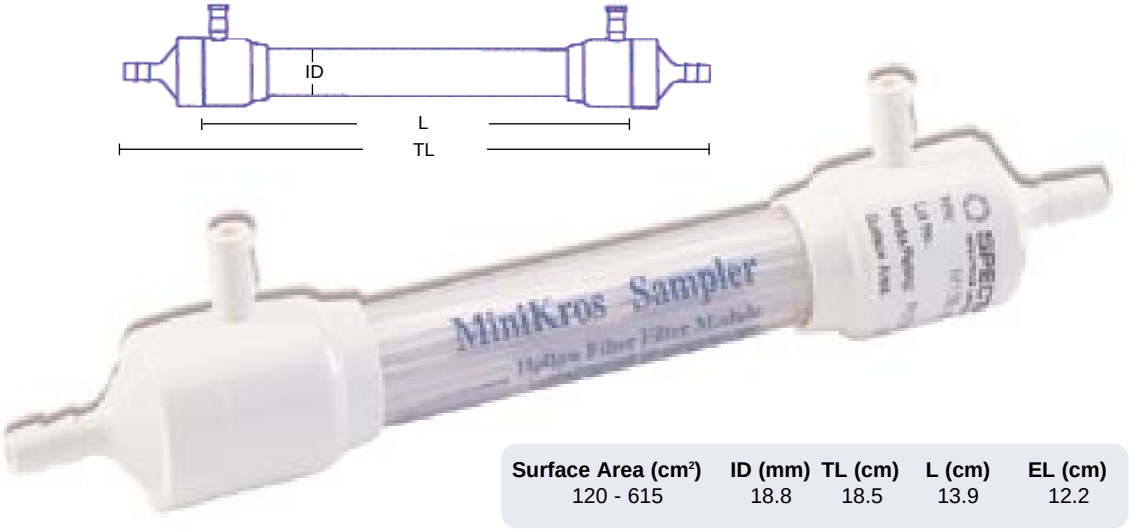
Table 2.1 - ACMS-400-01N Set-up Kit for MiiniKros® Sampler System

illustrations and figures on individual parts

illustrations and figures on individual parts

3. Modules Supported by the MiniKros® Sampler System

The MiniKros Sampler System is designed to allow Spectrum modules to be used for batch and topped-off batch separations, as well as constant volume diafiltrations where small volumes are desired. The system supports the following membrane modules which must be purchased separately.



Surface Area (cm²)	ID (mm)	TL (cm)	L (cm)	EL (cm)
120 - 615	18.8	18.5	13.9	12.2

Figure 3.1 - MiniKros Sampler module with process volumes from 50 ml to 4 liters and module dimensions.

3.1 MiniKros Sampler Modules Ordering Information

MiniKros® Sampler Modules for Small Volume Separations

Product Number	Description	Inlet/Outlet	Connections Side Ports	Rating	Surface Area	Fiber Diameter	Fiber Type
M15E 220 01N	MiniKros Sampler - dry	1/4HB	FLL	0.5 µm	120 cm²	0.5 mm	PES
M15E 260 01N	MiniKros Sampler - dry	1/4HB	FLL	0.5 µm	365 cm²	0.5 mm	PES
M15E 220 01S	MiniKros Sampler - dry,irr	1/4HB	FLL	0.5 µm	120 cm²	0.5 mm	PES
M15E 260 01S	MiniKros Sampler - dry,irr	1/4HB	FLL	0.5 µm	365 cm²	0.5 mm	PES
M15E 221 01N	MiniKros Sampler - dry	1/4HB	FLL	0.5 µm	95 cm²	1.0 mm	PES
M15E 261 01N	MiniKros Sampler - dry	1/4HB	FLL	0.5 µm	290 cm²	1.0 mm	PES
M15E 221 01S	MiniKros Sampler - dry,irr	1/4HB	FLL	0.5 µm	95 cm²	1.0 mm	PES
M15E 261 01S	MiniKros Sampler - dry,irr	1/4HB	FLL	0.5 µm	290 cm²	1.0 mm	PES
M12E 220 01N	MiniKros Sampler - dry	1/4HB	FLL	0.2 µm	120 cm²	0.5 mm	PES
M12E 260 01N	MiniKros Sampler - dry	1/4HB	FLL	0.2 µm	365 cm²	0.5 mm	PES
M12E 220 01S	MiniKros Sampler - dry,irr	1/4HB	FLL	0.2 µm	120 cm²	0.5 mm	PES
M12E 260 01S	MiniKros Sampler - dry,irr	1/4HB	FLL	0.2 µm	365 cm²	0.5 mm	PES
M12E 221 01N	MiniKros Sampler - dry	1/4HB	FLL	0.2 µm	95 cm²	1.0 mm	PES
M12E 261 01N	MiniKros Sampler - dry	1/4HB	FLL	0.2 µm	290 cm²	1.0 mm	PES
M12E 221 01S	MiniKros Sampler - dry,irr	1/4HB	FLL	0.2 µm	95 cm²	1.0 mm	PES
M12E 261 01S	MiniKros Sampler - dry,irr	1/4HB	FLL	0.2 µm	290 cm²	1.0 mm	PES

MiniKros® Sampler Modules for Small Volume Separations

Product Number	Description	Inlet/Outlet	Connections Side Ports	Rating	Surface Area	Fiber Diameter	Fiber Type
M12M 220 01N	MiniKros Sampler - dry	1/4HB	FLL	0.2 µm	155 cm²	0.6 mm	ME
M12M 260 01N	MiniKros Sampler - dry	1/4HB	FLL	0.2 µm	460 cm²	0.6 mm	ME
M12M 220 01S	MiniKros Sampler - dry,irr	1/4HB	FLL	0.2 µm	155 cm²	0.6 mm	ME
M12M 260 01S	MiniKros Sampler - dry,irr	1/4HB	FLL	0.2 µm	460 cm²	0.6 mm	ME
M12M 221 01N	MiniKros Sampler - dry	1/4HB	FLL	0.2 µm	150 cm²	1.0 mm	ME
M12M 261 01N	MiniKros Sampler - dry	1/4HB	FLL	0.2 µm	460 cm²	1.0 mm	ME
M12M 221 01S	MiniKros Sampler - dry,irr	1/4HB	FLL	0.2 µm	150 cm²	1.0 mm	ME
M12M 261 01S	MiniKros Sampler - dry,irr	1/4HB	FLL	0.2 µm	460 cm²	1.0 mm	ME
M11M 220 01N	MiniKros Sampler - dry	1/4HB	FLL	0.1 µm	155 cm²	0.6 mm	ME
M11M 260 01N	MiniKros Sampler - dry	1/4HB	FLL	0.1 µm	460 cm²	0.6 mm	ME
M11M 220 01S	MiniKros Sampler - dry,irr	1/4HB	FLL	0.1 µm	155 cm²	0.6 mm	ME
M11M 260 01S	MiniKros Sampler - dry,irr	1/4HB	FLL	0.1 µm	460 cm²	0.6 mm	ME
M10S 220 01N	MiniKros Sampler - dry	1/4HB	FLL	0.05 µm	245 cm²	0.5 mm	PS
M10S 260 01N	MiniKros Sampler - dry	1/4HB	FLL	0.05 µm	615 cm²	0.5 mm	PS
M10S 220 01S	MiniKros Sampler - dry,irr	1/4HB	FLL	0.05 µm	245 cm²	0.5 mm	PS
M10S 260 01S	MiniKros Sampler - dry,irr	1/4HB	FLL	0.05 µm	615 cm²	0.5 mm	PS
M10S 220 01P	MiniKros Sampler - wet	1/4HB	FLL	0.05 µm	245 cm²	0.5 mm	PS
M10S 260 01P	MiniKros Sampler - wet	1/4HB	FLL	0.05 µm	615 cm²	0.5 mm	PS
M14S 220 01N	MiniKros Sampler - dry	1/4HB	FLL	400 kD	245 cm²	0.5 mm	PS
M14S 260 01N	MiniKros Sampler - dry	1/4HB	FLL	400 kD	615 cm²	0.5 mm	PS
M14S 220 01S	MiniKros Sampler - dry,irr	1/4HB	FLL	400 kD	245 cm²	0.5 mm	PS
M14S 260 01S	MiniKros Sampler - dry,irr	1/4HB	FLL	400 kD	615 cm²	0.5 mm	PS
M14S 220 01P	MiniKros Sampler - wet	1/4HB	FLL	400 kD	245 cm²	0.5 mm	PS
M14S 260 01P	MiniKros Sampler - wet	1/4HB	FLL	400 kD	615 cm²	0.5 mm	PS
M15S 220 01N	MiniKros Sampler - dry	1/4HB	FLL	50 kD	245 cm²	0.5 mm	PS
M15S 260 01N	MiniKros Sampler - dry	1/4HB	FLL	50 kD	615 cm²	0.5 mm	PS
M15S 220 01S	MiniKros Sampler - dry,irr	1/4HB	FLL	50 kD	245 cm²	0.5 mm	PS
M15S 260 01S	MiniKros Sampler - dry,irr	1/4HB	FLL	50 kD	615 cm²	0.5 mm	PS
M15S 220 01P	MiniKros Sampler - wet	1/4HB	FLL	50 kD	245 cm²	0.5 mm	PS
M15S 260 01P	MiniKros Sampler - wet	1/4HB	FLL	50 kD	615 cm²	0.5 mm	PS
M11S 220 01N	MiniKros Sampler - dry	1/4HB	FLL	10 kD	245 cm²	0.5 mm	PS
M11S 260 01N	MiniKros Sampler - dry	1/4HB	FLL	10 kD	615 cm²	0.5 mm	PS
M11S 220 01S	MiniKros Sampler - dry,irr	1/4HB	FLL	10 kD	245 cm²	0.5 mm	PS
M11S 260 01S	MiniKros Sampler - dry,irr	1/4HB	FLL	10 kD	615 cm²	0.5 mm	PS
M11S 220 01P	MiniKros Sampler - wet	1/4HB	FLL	10 kD	245 cm²	0.5 mm	PS
M11S 260 01P	MiniKros Sampler - wet	1/4HB	FLL	10 kD	615 cm²	0.5 mm	PS

4. Basic Concept of Crossflow Separation

4.1 Membrane Separations

Membranes use the principle of barrier separations to differentiate components based on size. Components larger than the membrane pore are quantitatively held back by the membrane (retentate) while smaller components pass through the membrane structure along with the permeate (filtrate). Microgon MiniKros membranes are available in a wide range of pore sizes to serve all your microfiltration and ultrafiltration needs.

4.2 Dead Ended Separations

Traditional sieve (or dead ended) filtrations consist of forcing a solution containing suspended solids directly through the membrane structure. Solids retained by the membrane collect on the surface of the membrane media, continually reducing the permeation rate and eventually plugging the device. (See Figure 4.1).

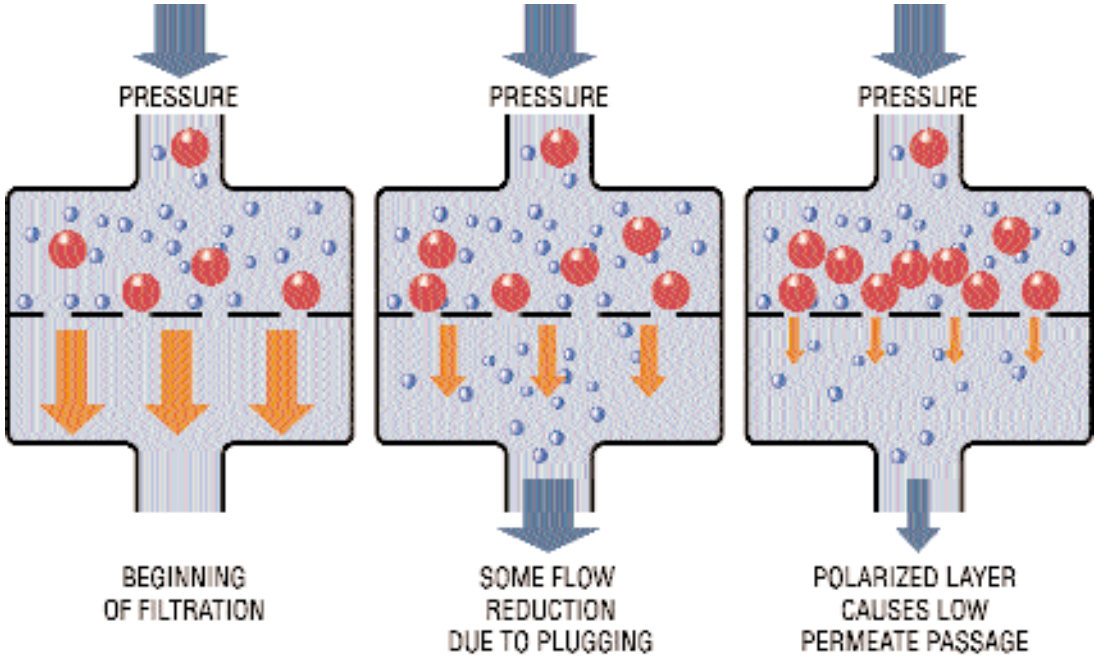


Figure 4.1 - Build up of polarized layer in dead end filtration mode.

The driving force for permeation is the pressure difference between the feed and the permeate, called the transmembrane pressure or TMP. For dead ended filtration this is given by the following equation:

Ptransmembrane = Pfeed - Ppermeate

4.3 Tangential Flow Separations

Tangential flow (or crossf low) separations are an efficient way of separating suspended solids from a'solution because the bulk of the solution flows parallel ~to the filter surface rather than perpendicular. to It. Particles, that would normally clogthe filter, are whisked up and recirculated. Crossflow velocitykeeps the.membiane clean, preventing the accumula-

tion of particles, cells, or debris on the membrane surface.

When using tangential flow techniques, most of the process fluid flows along the membrane surface rather than passing thru the membrane structure. A small portion.of the inlet flow (often less than 1%) permeates the membrane and exits the membrane as filtrate or “permeate” What remains of the inlet flow exits the module slightly, enriched in the components that are held in (or retained) by the membrane. This retained stream is called the “retentate”. As the retentate continuously recirculates back into the module and over the membrane, more filtrate is progressively excreted while the retentate is progressively concentrated. (see Figure 4.2).

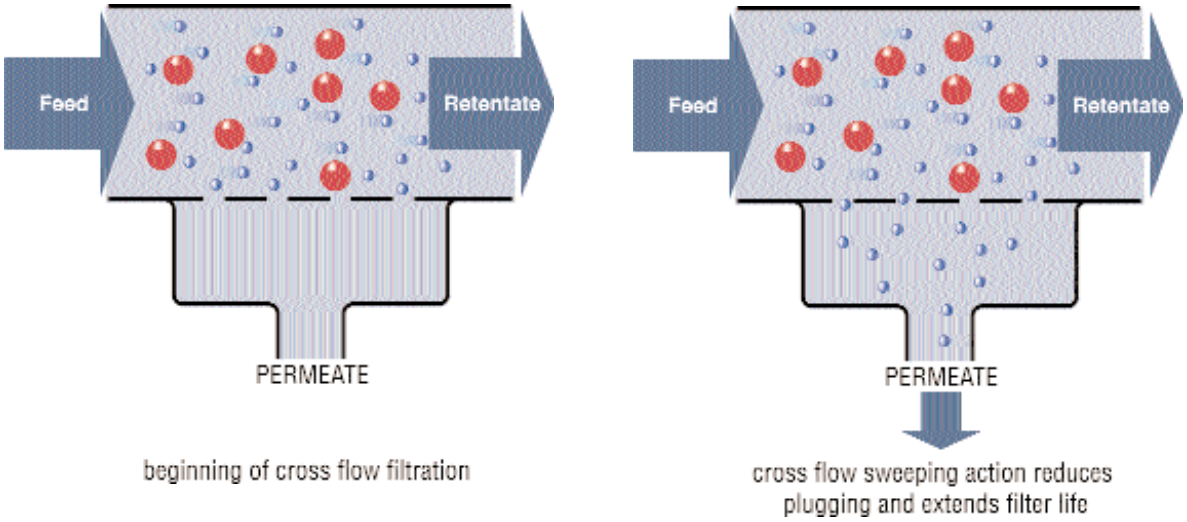


Figure 4.2 - Cross Flow pde reduces concentration polarization and extends filter life

Various tangential flow membrane geometries include: stacked plate and spiral devices which utilize flat sheet membranes; tubular devices; as well as shell and tube devices which use hollow fiber membranes.

Microgon Sampler Modules are made of hollow fiber bundles encased in a. shell housing. The process fluid flows down the length of the fibers ~at high velocity.. Perm~ate seeps through the pores of the membrane, into the module housing, and out one of the two filtrate ports. The remaining process fluid or retentate exists the module and is recircUlated until all the particles smaller than the membrane pores are in the filtrate and all the particles larger than the membrane pores are in the ‘retentate. (see
Figure 4.3, next page)

Hollow fiber membranes have the distinct advantage of providing the largest filter surface areas for a given fluid volume. MiniKros hollow fiber modules come in a wide ‘range of surface area for effective scale up. In addition, multiple hollow fiber modules set up in parallel provide another strait forward method of scaling up.

In the case of tangential flow separations, the driving force (transmembrane pressure or TMP) is the difference between the average of the membrane feed and retentate pressures, minus the permeate pressure:

Ptransmembrane = (Pfeed + Pretentate) /2 Ppermeate

Filtrate flow results in a build up of retained components on the membrane inside surface.

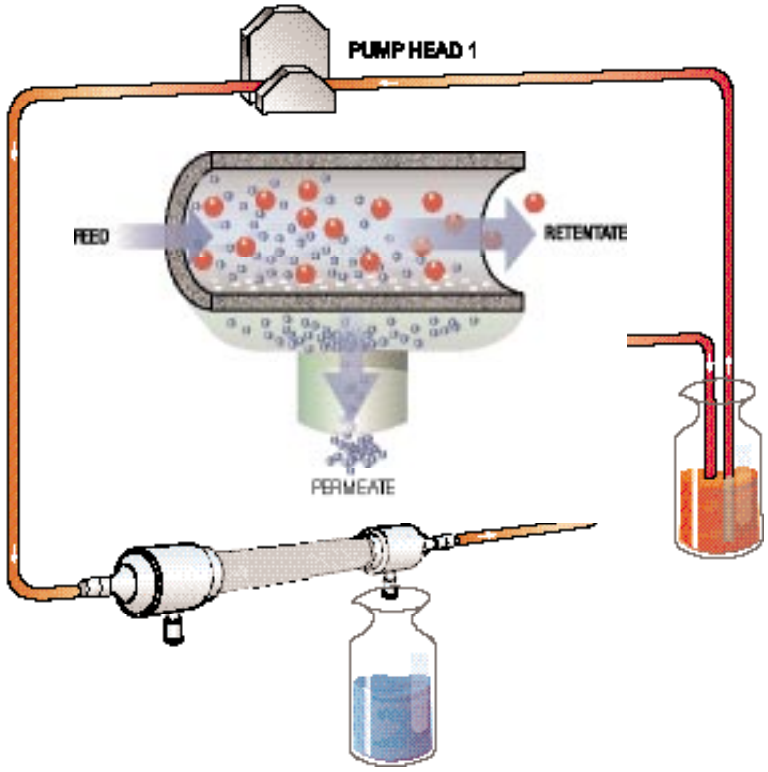


Figure 4.3 - Diagram of typical hollow fiber filtration set up.

Generally these components are carried down the length of the membranes and out the end of the module by the sweeping action of the recirculating fluid. However, under certain conditions a gel layer accumulates on the surface of the membrane. This boundary layer is composed of solids and/or solute macromolecules which are retained by the membrane during the course of filtration. This phenomenon, often erroneously called 'concentration polarization' can affect module performance by reducing the apparent size of the membrane pore. In other words, the g& layer becomes the membrane barrier, a 'dynamic membrane'.

The gel layer is influenced by such fluid variables as the degree of solvation, concentration and nature of the solids and solutes, fluid temperature, and operating variables such as solution velocity along the membrane and transmembrane pressure (TMP). Controfling these phenomena is key to maximizing flux and solute passage.

The MiniKros Sampler System provides a unique environment for optimizing the velocity and transmembrane pressure conditions as well as economically matching process fluids with their appropriate module. The MiniKros Sampler System works wjth small volumes thus providing an inexpensive means for optimization and experimentation. Once conditions have been optimized on the Sampler System then scale-up is a straight forward and predictable process.

Spectrum tangential flow membrane modules differ from traditional tangential flow membrane devices in that they are supplied non-pyrogenic, ready for use, and are priced to be disposable. Cleaning chemicals do not have to be rinsed from the membrane after cleaning. Also, repeated and exhaustive cleaning studies are not needed to guarantee scale-up and predictability. Using a fresh, disposable MicroKros Modules ensures consistent results and higher yields.

5. Modes of Operation:

The Sampler System mimics large. scale applications while processing very small test volumes. This kind of low volume versatility is useful for doing pilot research studies where limited volumes of processfluids are available.

5.1 Batch Operations

Batch operation recirculates retentate from the module directly back into the feed tank. As filtrate is collected materials retained by the membrane become progressively more concentrated and the level of the feed tank drops. The advantage of batch operation is that the filter is only exposed to~~~high áánôentration late in the run. The disadvantage of batch operation is that vortexing is likely to occur when low volumes are reached in the feed tank. The batch mode is best utilized for large processing volumes where highest efficiency is desired early in the run.

Batch Concentration (Retentate is the product)

Generally, the term concentration refers to applications where the material retained by the membrane is (or contains) the desired product. As the process fluid is recirculated through the membrane, there is a reduction of volume in the feed tank due to the removal of permeate. As a result, the product is concentrated in the feed tank. This mode of operation is used, for example, with fermentation recoveries where the desired product is the cell itself or as an initial processing step where product is intracellular.

Batch Clarification (Permeate is the product)

The term clarification is generally used for applications where the desired product is in the permeate. For example this mode of operation may be used with a microporous membrane to harvest animal cell fermentations where desired product is secreted by the cells or released into solution by lysing of the cells.

Concentration Factor

When operated in concentration or clarification modes, me membrane quantitatively removes solids larger than the pores of the membrane and allows the passage of soluble materials that are smaller than the membrane pores.

As permeate is removed, the process vessel become more concentrated in the components that the membrane retains and volume is reduced. The degree of concentration, called the concentration factor (CF) or volume reduction factor (VRF) is given by the following equation where Vi is the initial volume and Vf is the final volume:

Concentration Factor = Volume Reduction Factor = Vi / Vf

5.2 Constant Volume Operations — Using a reservoir

Batch operations work very well until the feed tank reaches very low volumes. At that point the process fluid often vortexes or foams in the feed tank due to the high recirculation rate (20 to 100 times the permeate rate). A way of avoiding this problem is to operate the processing vessel at constant volume. This can be achieved by adding a reservoir to the system. The reservoir is kept at constant volume by adding processing fluid from the feed tarjk at the same rate that permeate is removed. This technique is called topped-off batch operation.

Take the example of harvesting a fermenter or s~rred-tank bioreactor. Users often attempt to use the bioreactor vessel as the feed tank, with poor results: the fermenter exit and entrance ports are frequently too small for adequate recirculation flow, the return line must be modified so that it is submerged at high concentrations (low volumes). Foaming or vortexing frequently occurs with relatively large amounts of the broth still left un-processed (lower than desired concentration factors). In addition, shear due to foaming can damage and/or denature the product.

Constant volume operation is useful for both concentration and clarification where it is desired to eliminate the possibility vortexing in the feed tank. The disadvantage of constant volume filtration is that the reservoir fluid concentration increases more rapidly as additional feed is adci'to the reservoir. However this is not a concern when using constant volume operation for diafiltration or washing because the fluid added to the reservoir is washing buffer and free of solids, etc. (see below).

Diafiltration or Washing - Addition of extra buffer solution

Materials that pass thru the membrane can be washed away from materials that are retained by the membrane (cells, particles, etc.). It also can be used to wash salts away from proteins when protein retentive (UF) membranes are used. The technique is used to recover additional product in clarification applications, and to achieve better product purity in concentration applications. For best efficiency, the wash buffer should be free of the solute that is being recovered or removed.

Diafiltration may be accomplished either by adding buffer at the same rate as the permeation rate with a reservoir (constant volume diafiltration), or by reducing the volume in the feed tank and re-adding buffer to regain the original volume (discontinuous diafiltration). The amount of diafiltration performed can be expressed by the amount of wash buffer added divided by the batch volume, i.e. the number of 'wash volumes'.

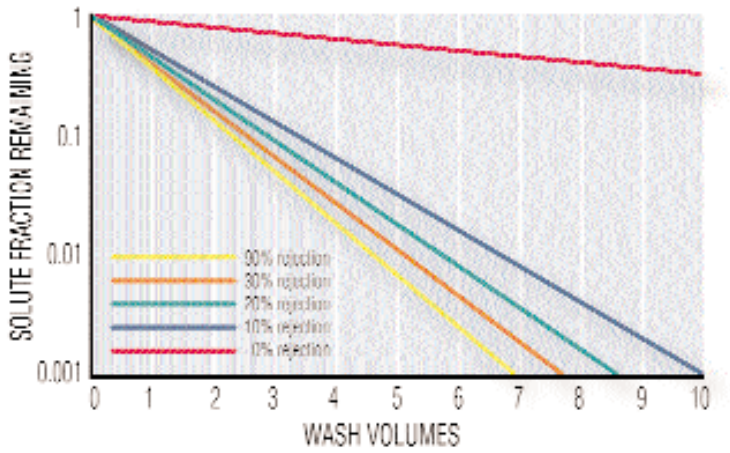


Figure 5.1 - Effect of solute rejection on constant volume diafiltration

During a constant volume diafiltration where soL~ble components pass freely thru the membrane, each wash volume of permeate removed reduces the solute concentration by factor of e (2.718..). For example, a four volume constant volume diafiltration will reduce the concentration of solute by a factor of e4, i.e. 50-fold or over 98%. Using this technique, the concentration of solute can be monitored in the permeate until the desired level of purification or product recovery is achieved. Components that are partially retained by the membrane cannot be diafiltered or washed with the same efficiency (see Figure 5.1). The membrane rejection (or retention) is defined as:

$$\text{Rejection} = 1 - \frac{\text{Concentration in the permeate}}{\text{Concentration in the retentate}}$$

5.3 Minimum Volume Operations — For lowest possible hold-up volumes

Minimum volume operation is for situations where hold-up volume needs to be kept very low. Minimum volume operation is similar to constant volume operation except that the tubing is used as the reservoir. Retentate recirculates in the loop of tubing containing the module and the pump. As the filtrate exits the module new process fluid is drawn into the tubing loop from the feed tank. In this way hold-up volumes of 18 ml can be achieved with MiniKros modules and as low as 2 ml with MicroKros modules.

The negative aspect of minimum volume operations is that there is no vessel present for deaeration. Care must be taken to prevent air bubbles from entering the recirculation loop

5.4 Dead Ended Operation — For enhanced permeate recovery

While the normal mode of operation involves tangential flow, Spectrum crossf low membrane modules can also be run dead ended. In the dead ended mode, the retentate line (exiting the module) is capped or blocked by a hose shut-off clamp so that all of the solution being processed passes through the membrane wait. It is possible to initially utilize a tangential mode to recover permeate then switch to a dead end mode to enhance product recovery (squeeze out every last drop). Though the efficiencies of tangential flow separation are lost, in certain circumstances such as at the end of a run where maximum permeate recovery is desired, running dead ended can be advantageous.

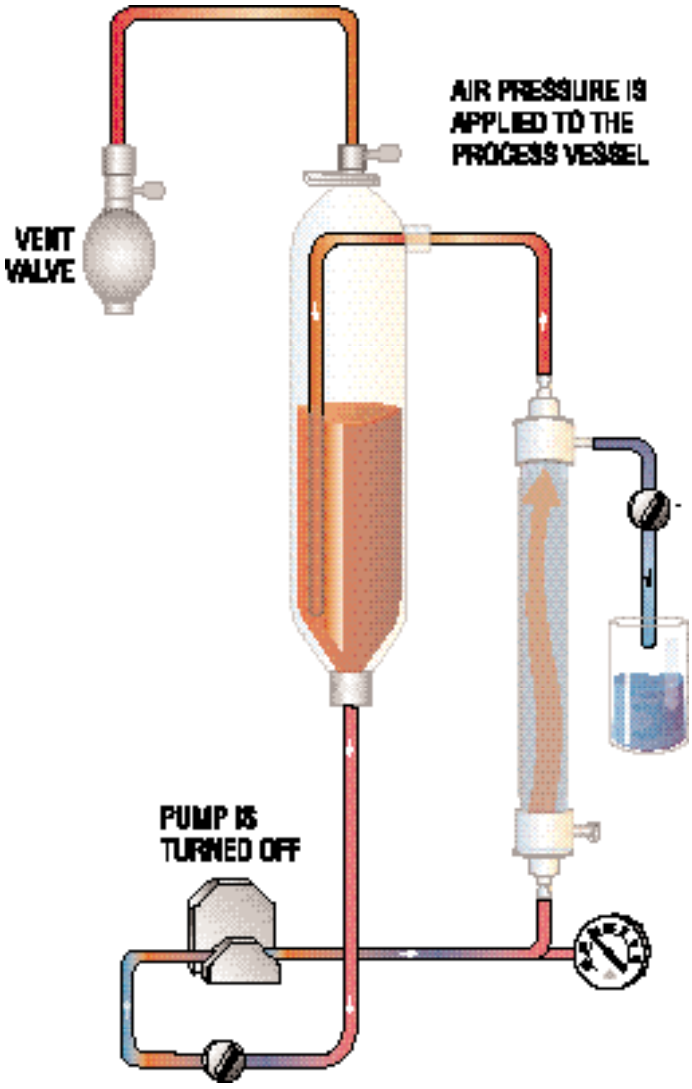


Figure 5.2 - Dead Ended operation

Conventional tangential flow membrane devices, which have high membrane costs, must be cleaned and re-used to be economical; dead ended techniques are avoided because they interfere with membrane cleaning. Spectrum disposable membranes, however, lend themselves to this mode of product recovery. Since membrane investment is comparatively low, it is not necessary to clean a Spectrum module subsequent to use and dead ended techniques can be economically useful.

6. Batch Assembly for MiniKros® Sampler System (Figure 6.1)

1. Cut two 1 cm lengths of the thin wall silicone tubing (not size 16 tubing).
2. Push thin wall tubing half way over the following items:
   • The retentate hose barb fittings of the MiniKros Sampler Module.
   • One 'female Luer to 5 mm hose barb adaptor'.
3. Connect the female Luer adaptor with the silicone tubing sleeve to the stem (base) of a Y connector so as to make a secure seal.
4. Connect the MiniKros Module hose barb fitting with the silicone tubing sleeve into an arm of the Y connector so as to~make a secure seal.
5. Cut 26 cm of size 17 tubing and connect one end of the tubing to the V connector. Secure the tubing with a size 4 hose clamp (very important since this connection will be pressurized).
6. Connect the transducer to the female Luer adaptor of the Y connector. To the other end of thetransducer connect the Luer check valve. On the end of the Luer check valve connect a male Luer cap.
7. Cut one length of size 16 silicone tubing. Connect the size 16 tubing from the remaining hose barb of the MiniKros module to one of the hose barbs of the 2 liter feed tank.
8. Connect a 'female Luer toii5...rnm hOse barb adaptor' to the remaining end of the size 17 tubing and secure with a size 4 hose clamp.
9. Cut two lengths of size 16 silicone tubing. Install a 'male Luer to 3 mm hose barb adaptor' on each tube segment and secure with size 1 hose clamps.

   • Connect the male Luer of the size 16 silicone tubing to one of the filtrate ports of the MiniKros Module (it does not matter which filtrate port, MiniKros Modules are symmetrical, though it is helpful for the label to face out). Slide a hose shut-off clamp on the size 16 tubing.

   • Connect the male Luer end of the remaining size 16 silicone tubing to the female Luer connector of the size 17 tubing (this one will become the feed or buffer inlet connection).
10. Direct the size 16 silicone tubing from the filtrate port of the MiniKros Module to a measured collection vessel (not supplied).
11. Cut 2 cm from one of the pieces of hard polypropylene tubing and connect to the inflation bulb.
12. Remove the size 17 silicone tubing froniiti~ filling~~n ci re~Lthe 2 liter buffer ié~eW~i~ikinstall two segments of polypropylene tubing to the two inside recept~~i~ ti1lt~feed tank closures. Connect the size 16 silicone tubing from size 17 tubing to the 2 liter feed tank hose barb fitting that leads to the submersion tube.
13. Connect the 2 cm of polypropylene tubing from the infation bulb to the 2 liter feed tankvent fitting.
14. Install the module holder clips to the pump using the two screw heads that insert into the pump head (the older style white clips may require manipulation and bending). If your system has the white metal clips, call Microgon to request a complementary upgrade.

15. Place MiniKros Sampler Module into the holder clips with the filtrate oittl~t directed upward (best to have the Microgon label facing outward). Adjust the position of all tubing and connectors as necessary.

16. Place the size 17 tubing into the pump head (over the rollers and into the groove). Rotate the locking arm clockwise to lock the size 17 tubing into place. Adjust the black clips on the pump head for holding the tubing in place so that the tubing does not pull through the pump head during operation. Keep the pump head open when not in use so as to not impart umemoryu to the tubing.

figure 6.1 - batch assembly for minikros sampler system

7. Batch Protocol for Operating the MiniKros® Sampler System

7.1 Testing for Leaks in the System

The seals of system setup should be pressure tested with air before filling with fluids.

- Close the shut-off clamp on the filtrate outlet line.
- Turn on the pressure monitor and tare it to zero.
- Use the inflation bulb of the feed tank to reach a pressure of approximately 5 psi.
- If the pressure drop is less than 0.1 psi/mm. then the system is usable.
- If the pressure drop is greater than 0.1 psi/mm. then check to make sure all the seals are secure. Adjust seals until pressure drop is reduced.
- Relieve the pressure by momentarily opening the vent on the inflation bulb.

7.2 Filling the System with Fluid

- Add water/buffer to the 2 liter feed tank.
- Pump the inflation bulb on the 2 liter feed tank.
- Once fluid begins circulating into the system then twist the vent valve of the inflation bulb to relieve pressure.
- Establish a pump velocity (corresponding to approximately 5 psi). This should force out the remaining air pockets.

7.3 Pre-Rinsing the Module

A minimum of 1 ml water/buffer solution per cm2 of membrane area should be filtered to eliminate residual glycerin from the membrane prior to introducing product.

- Establish a pump velocity (corresponding to approximately 5 psi).
- Open the shut-off clamps for filtrate tube.
- Collect appropriate volume of water/buffer filtrate to achieve 1 mVcm2.

Note: Any time that the system is filled with fluid and the pump is turned off then the shut-off clamp for the filtrate tubes should be locked. Because the retentate volume is small, pressure without recirculation can plug the membrane. Although there is..no pressure due to pumping, hydrostatic pressure can make the filter permeate in a dead end mode.

7.4 Filtration Processing of Fluid

- The membrane inlet pressure and velocity of process tuid should be adjusted for each specific application. Transmembrane pressure can be increased by increasing the recirculation velocity, pressurizing the feed vessel, or pulling a vacuum on the filtrate line.
- Generally, the membrane inlet pressure of should be 5 psi for microfiltration and 10 psi for ultrafiltration. Membrane inlet pressure should not exceed 20 psi, the maximum transducer pressure.
- Additional transmembrane pressure will frequently speed up ultrafiltration separations without adverse effects. Additional transmembrane pressure above 20 psi can only be

achieved by pulling a vacuum on the permeate. -

- As fluid in the 2 liter feed tank lowers to a volume of approximately 200 ml there may be vortexing and bubbling. The remaining fluid in the feed tank should be removed from the feed tank and processed in an alternative method (such as constant volume or minimum volume mode of operation).
- Operating conditions should be recorded using the performance log. (provided in appendix A)

open for suggestions

8. Constant Volume 60 ml Reservoir Assembly for MiniKros® Sampler System. (see figure 8.1)

1. Cut four 1 cm lengths of the thin wall silicone tubing (not size 16 tubing).
2. Push thin wall tubing half way over the following items:
   - One of the retentate hose barb fittings of the MiniKros Module.
   - Two of the female Luer to 5 mm hose barb connectors.
   - One of the hose barb fittings of the 60 ml reservoir (ACTO-P06-01 N).
3. Connect the two female Luer adaptors w~h the silicone tubing sleeve into the stem (base) of the two clear Y connectors so as to make a secure seal.
4. Connect the transducer to the female Luer of one of the Y connectors (which will now be designated as the “transducer Y connector”).
5. Push the MiniKros Module hose barb fitting with the silicone tubing sleeve into an arm of the” transducer Y connector” so as to make a secure seal. (This will be the high pressure inlet to the MiniKros Module.)
6. Cut 26 cm of size 17 tubing and connect to the remaining arm of the “transducer V connector”. Secure the tubing with.a size 4 hose clamp. (Make sure that the seal is secure because this V connector will be the highest point of pressure in the system.)
7. The remaining V connector will be designated as the ‘vessel V connector”.
   - Push one of the open arms of the ‘vessel Y connector’ over the hose barb (with the thin silicone tubing sleeve) of the 60 ml reservoir.
   - Secure the remaining open arm of the ‘vessel Y connector” into the size. 17 tubing and secure with size 4 hbse clamp.
8. Cut one length (approximately 14 cm) of size 16 silicone tubing to connect the remaining retentate hose barb fitting of the MiniKros Module to the remaining hose barb fitting of the reservoir.
9. The remaining fitting of the 60 ml reservoir is the air vent reservoir. Seal the reservoir air vent with one of the caps (clear or black) taken from the 2 liter vessel.
10. Cut two lengths of size 16 silicone tubing. Install a male Luer by 3 mm hose barb adaptor on each tube segment and secure with size 1 hose clamps. Slide a hose shutoff clamp on the Luer fitting end of each length of tubing.
    - Connect the male Luer of the size 16 silicone tubing to one of the filtrate ports of the MiniKros Module (it does not matter which one, MiniKros Modules are symmetrical, though it is helpful for the label to face out).
    - Connect the male Luer end of the remaining size 16 silicone tubing to the female Luer connector on the “vessel Y connector” (this one will become the feed or buffer inlet connection).
11. Direct the size 16 silicone tubing from the filtrate port of the MiniKros Module to a measured collection vessel (not supplied).
12. Cut 2 cm from the piece of hard polypropylene tubing and connect to the inflation bulb.
13. Remove the silicone tubing from the fil!inglventing closure of the 2 liter buffer feed tank.

Install the remaining segment of polypropylene tubing to the inside of the 2 liter feed tank closure. Connect the size 16 silicone tubing from the ~‘ vessel Y connector” to the 2 liter feed tank hose barb fitting that leads to the submersion tube.

14. Connect the 2 cm of polypropylene tubing from the inflation bulb to the 2 liter feed tank vent fitting. Cap the remaining hose barb fitting of the feed tank with a cap.
15. Install the module holder clips to the pump using the two screw heads that insert into the pump head (the older style white clips may require manipulation and bending). If your system has the white metal clips, call Microgon to request a complementary upgrade.
16. Place MiniKros Sampler Module into the holder clips with the filtrate outlet directed upward. Adjust the position of all tubing and connectors as necessary.
17. Place the. size 17 tubing into the pump head (over the rollers and into the groove). Rotate the locking arm clockwise to lock the size 17 tubing into place. Adjust the black clips on the pump head for holding the tubing in place so that the tubing does not pull through the pump head during operation. Keep the pump head open when not in use so as to not impart Nmemoryu to the tubing.

figure 8.1 - constant volume 60 ml reservoir assembly for MiniKros sampler syst

9. Constant Volume 60 ml Reservoir Protocol for the MiniKros Sampler System

9.1 Testing for Leaks in the System

The seals of system setup should be pressure tested with air before filling with fluids.

- Close the shut-off clamp on the filtrate outlet line.
- Turn on the pressure monitor and tare it to zero.
- Use the inflation bulb of the feed tank to reach a pressure of approximately 5 psi.
- If the pressure drop is less than 0.1 psi/mmn. then the system is usable.
- If the pressure drop is greater than 0.1 psi/mm. then check to make sure all the seals are secure. Adjust seals until pressure drop is reduced.
- Relieve the pressure by momentarily opening the vent on the inflation bulb.

9.2 Filling the System with Fluid

- Fill the 2 liter feed tank with appropriate qual~ty water or washing buffer.
- Open the shut-oft clamps for both the feed and filtrate tubes.
- Remove the vent cap of the 60 ml reservoir.
- Pump the inflation bulb to the 2 liter feed tank, so as to push bufter into the 60 ml reservoir.
- Once the 60 ml reservoir has filled then cap the vent of the 60 ml reservoir.
- Relieve the pressure on the 2 liter feed tank by momentarily opening the vent on the inflation bulb.
- Turn the pump on forward rotation and increase the vebcity until a flow pressure is generated of approximately 5 psi. At this point air pockets will be forced through the system and will collect in the 60 ml reservoir thus lowering the reservoir fluid level.
- Before shutting off the pump, always close the hose shut-off clamps for both the feed and the filtrate tubes.

Note: Any time that the system is filled with fluid and the pump is turned off then the shut-off clamps for both the feed and the filtrate tubes should be locked. Because the retentate volume is small, pressure without recirculation can plug the membrane. Although there is no pressure due to pumping, hydrostatic pressure can make the filter permeate in a dead end mode.

9.3 Adjusting Fluid Level of 60 ml Reservoir

For the procedures that follow it will be necessary to adjust the fluid level of the 60 ml reservoir.

Lowering the fluid levels of the 60 ml reservoir:

- Establish a pump velocity (corresponding to approximately 5 psi)
- Close the feed shut-off clamp.

- Open the filtrate shut-off clamp.
- Open the 60 ml reservoir vent by lifting the cap.
- When desired reservoir volume is reached, close the filtrate shut-off clamp and re-apply the cap to the reservoir vent. Avoid going so low as to cause vortexing and bubbling in the 60 ml reservoir chamber.
- Open feed shut-off clamp.

Raising the fluid levels of the 60 ml reservoir:

- Establish a pump velocity (corresponding to approximately 5 psi)
- Close the filtrate shut-off clamp.
- Open the feed shut-off clamp.
- Open the 60 ml reservoir vent by lifting the cap (fluid levels may rise quickly).
- When desired reservoir volume is reached then re-apply the cap to the reservoir vent.
- Open filtrate shut-off clamp.

Note: Whenever 60 ml reservoir volume fills or drains with the reservoir vent closed, then there is an air leak in the system.

9.4 Pre-Rinsing the Module

A minimum of 1 ml water/buffer solution per cm2 of membrane area should be filtered to eliminate residual glycerin from the membrane prior to introducing product.

- Establish a pump velocity (corresponding to approximately 5 psi).
- Establish a 60 ml reservoir chamber level of approximately 50 ml.
- Open the shut-off clamps for both the feed and filtrate tubes.
- Collect appropriate volume of water/buffer filtrate to achieve 1 ml/cm2 (200 ml for module listed above).
- Remove water/buffer from system (see Draining Fluid from System section).

9.5 Draining Fluid from System

- Lower the fluid level in the 60 ml reservoir chamber to approximately 20 ml
- Close the feed and filtrate shut-off clamps.
- Open the lid off the 60 ml reservoir container. Holding the lid above the 60 ml reservoir, use the pump to pump the remaining fluid from the system tubing into reservoir container.
- Shut-off the pump once the remaining fluid has emptied (and the pump is only pushing air).

9.5 DiafUtration

- Fill 60 ml reservoir with the process fluid to be washed.

- Follow the “Filling the System with Fluid” directions.
- Establish a pump velocity (corresponding to approximately 5 psi).
- The membrane inlet pressure and velocity of process fluid should be adjusted for each specific application. Transmembrane pressure can be increased by increasing the recirculation rate, pressurizing the feed tank, or pulling a vacuum on the filtrate line.
- Generally, the membrane inlet pressure of should be 5 psi for microfiltration and 10 psi for ultrafiltration. Membrane inlet pressure should not exceed 20 psi, the maximum transducer pressure.
- Additional transmembrane pressure will frequently speed up ultrafiltration separations without adverse effects. Additional transmembrane pressure above 20 psi can only be achieved by pulling a vacuum on the permeate.
- Adjust the 60 ml reservoir volume to a desired level (anywhere from 25 to 60 ml).
- Open the shut-off clamps for both the feed and filtrate tubes.
- Use the performance log to maintain a record of the operating conditions (see Appendix A).
- After the desired number of washings have been achieved the system can be drained.
- If it is desireable to recover the maximum yield of retained process fluid, the following technique can be used to maximize the particle recovery:
  - Begin concentration of the retentate by using the instructions for lowering the fluid level in the 60 ml reservoir.
  - Stop concentration when the fluid level reaches approximately 1 cm. (our goal is to avoid foaming or vortexing of the retentate).
  - Forward Flush the system by closing the feed and filtrate shut-off clamps. Run the pump at a reduced speed for 30 seconds in each direction. (see Appendix B for details)
  - Remove the retentate from the system by using the Draining Fluid from System” instructions. (The retentate volume should be half of the original volume. An equivalent volume will be flushed from the system in the next steps.)
  - To remove residual retentate from the system, add more water/buffer to the system by following the “Filling the System with Fluid” instructions.
  - Lower the level of the, fluid to approximately 1 cm as before.
  - Forward Flush the system a second time by closing the feed and filtrate shut-off clamps. Run the pump at a reduced speed for 30 seconds in each direction. (see Appendix B)
  - Remove the retentate from the system by using the Draining Fluid from SystemN instructions.
  - Combine the two washed volumes of retentate.
  - Additional rinses can be employed to gain additional recovery (though retentate will be diluted).

10. Minimum Volume Assembly for MiniKros® Sampler System Figure 10.1

1. Cut four 1 cm lengths of the thin wall silicone tubing (not size 16 tubing).
2. Push thin wall tubing half way over the following items:
   - The two retentate hose barb fittings of the MiniKros Sampler Module.
   - The two 'female Luer to 5 mm hose barb adaptors'.
3. Connect the two female Luer adaptors wfth the silicone tubing sleeve to the stem (base) of the two V connectors so as to make a secure seal.
4. Connect the MiniKros Module hose barb fittings with the silicone tubing sleeve into an arm of each Y connector so as to make a secure seal.
5. Orient the open remaining arms of the Y connectors so that they point in the same direction from the MiniKros Module.
6. Cut 26 cm of size 17 tubing and connect each end of the tubing to the two aligned arms of the Y connectors. Secure the tubing with size 4 hose clamps (very important since one of these connections will be pressurized).
7. Connect the transducer to the female Luer adaptors of one of Y connectors. This will become the high pressure end of the module. To the other end of the transducer connect the Luer check valve. One the end of the Luer check valve connect a male Luer cap.
8. Cut two lengths of size 16 silicone tubing. Install a male Luer by 3 mm hose barb adaptor on each tube segment and secure with size 1 hose clamps. Slide a hose shutoff clamp on the Luer fitting end of each length of tubing.
   - Connect the male Luer of the size 16 silicone tubing to one of the filtrate ports of the MiniKros Module (it does not matter which filtrate port, MiniKros Modules are symmetrical, though it is helpful for the label to face out).
   - Connect the male Luer end of the remaining size 16 silicone tubing to the female Luer connector of the remaining Y connector (this on will become the feed or buffer inlet connection).
9. Direct the size 16 silicone tubing from the filtrate port of the MiniKros Module to a measured collection vessel (not supplied).
10. Cut 2 cm from the piece of hard polypropylene tubing and connect to the inflation bulb.
11. Remove the size 17 silicone tubing from the filling/venting closure of the 2 liter buffer feed tank. Install the remaining segment of polypropylene tubing to the inside of the 2 liter feed tank closure. Connect the size 16 silicone tubing from the V connector to the 2 liter feed tank hose barb fitting that leads to the submersion tube.
12. Connect the 2 cm of polypropylene tubing from the inflation bulb to the 2 liter feed tank vent fitting. Cap the remaining hose barb fitting of the feed tank with a cap.
13. install the module holder slips to the pump using the two screw heads that insert into the pump head (the older style white clips may require manipulation and bending). If your system has the white metal clips, call Microgon to request a complementary upgrade.

14. Place MiniKros Sampler Module into the holder clips with the filtrate outlet directed upward. Adjust the position of all tubing and connectors as necessary.

15. Place the size 17 tubing into the pump head (over the rollers and into the groove). Rotate the locking arm clockwise to lock the size 17 tubing into place. - Adjust the blacl~ clips on the pump head for holding the tubing in place so that the tubing does not pull through the pump head during operation. Keep the pump head open when not in use so as to not impart '~memory" to the tubing.

figure 10.1 - minimum volume assembly for minikros sampler system

11. Minimum Volume Protocol for Operating the MiniKros® Sampler System

11. Testing for Leaks in the System

The seals of system setup should be pressure tested with air before filling with fluids.

- Close the shut-off clamp on the filtrate outlet line. -
- Turn on the pressure monitor and tare it to zero.-
- Use the inflation bulb of the feed tank to reach a pressure of approximately 5 psi.
- If the pressure drop is less than 0.1 psVmin. then the system is usable.
- If the pressure drop is greater than 0.1 psi/mm. then check to make sure all the seals are secure. Adjust seals until pressure drop is reduced.
- Relieve the pressure by momentarily opening the vent on the inflation bulb.

11.2 Filling the System with Fluid

- Remove the tubing and the MiniKros Module from the pump head.
- Open the shut-off clamps for both the feed and filtrate tubes.
- Pump the inflation bulb of the 2 liter feed tank.
- Orient the tubing setup vertically to eliminate air pockets.
- Remove the Luer check valve. Orient the tubing to allow excess air pockets to escape.
- Reconnect the Luer check valve to the transducer.
- Close both the filtrate and feed shut-off clamps.
- Install the MiniKros Module and the tubing back into the pump head.

11.3 Pre-Rinsing the Module

A minimum of 1 ml water/buffer solution per cm2 of membrane area should be filtered to eliminate residual glycerin from the membrane prior to introducing product.

- Establish a pump velocity (corresponding to approximately 5 psi).
- Open the shut-off clamps for both the feed and filtrate tubes.
- Collect appropriate volume of. water/buffer filtrate to achieve 1 mVcm2~

Note: Any time that the system is filled with fluid and the pump is turned off then the shut-off clamps for both the feed and the filtrate tubes should be locked. Because the retentate volume is small, pressure without recirculation can plug the membrane. Although there is no pressure due to pumping, hydrostatic pressure can make the filter permeate in a dead end mode.

11.4 Filtration Processing of Fluid

- The process fluid can be introduced into the system via the feed tank or through the check valve which may be used as a feed port.

- Overall processing volume is equal to the internal holdup volume of the tubing plus the MiniKros Module retentate volume. With a 26 cm loop the retentate volume is 18 ml.
- The membrane inlet pressure and velocity of process fluid should be adjusted for each specific application. Transmembrane pressure can be increased by increasing the recirculation velocity, pressurizing the feed vessel, or pulling a vacuum on the filtrate line.
- Generally, the membrane inlet pressure of should be 5 psi for microfiltration and 10 psi for ultrafiltration. Membrane inlet pressure should not exceed 20 psi, the maximum transducer pressure.
- Additional transmembrane pressure will frequently speed up ultrafiltration separations without adverse effects. Additional transmembrane pressure above 20 psi can only be achieved by pulling a vacuum on the permeate.
- Operating conditions should be recorded using the performance log. (provided in appendix A)

Appendix A. Sample Performance Data Log

Page _____ of_____

Date: _____

Company: _____ Contact: _____

Address: _____ Lot: No.: _____

_____ Phone: _____

Product: _____ Pump Type: _____

System Configuration: _____

Module Part and SN: _____

Initial Batch Volume: _____

Comments: _____

clock time	elapsed time	pressures (psig) P_{in}	P_{out}	$P_{filtrate}$	filtrate volume (ml)	seconds ml	temperature °F/°C	recirculation rate

Appendix B

Forward Flushing

Shutting the permeate while recirculating sets up a backflushing condition in the downstream half of the module. Shutting the permeate causes the permeate pressure to rise and exceed the retentate pressure in the downstream half of the module. In this region the permeate will flow from the outside of the membrane to inside. This loosens and carries away cake material. Normally forward flushing for 30 seconds is sufficient to clean the downstream half of the membranes.

The principle of forward flushing can be explained as follows: when the permeate is shut-off, the net permeation rate in the module is zero. But permeation still occurs internally: the inlet half of the membrane module (the high pressure end) generates permeate that back-ilushes the downstream half of the membrane module (the low pressure end). This phenomenon is called Sterling Flow. (see Figure B.1)

Reversing the pump with the permeate closed will serve to backflush the other half of the module and is an effective cleaning technique.

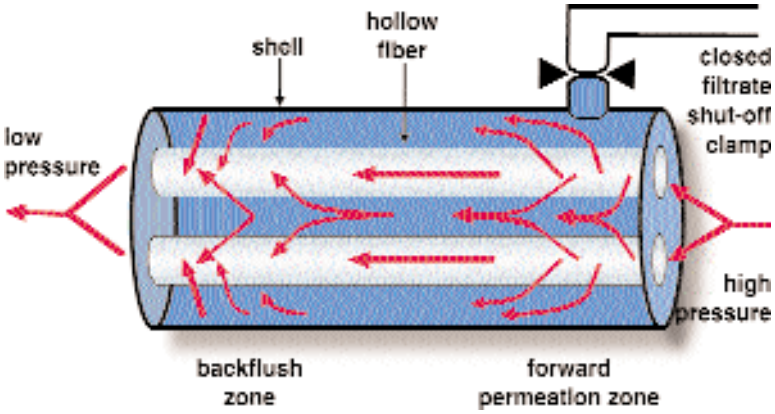


Figure B.1 - Forward Flushing of Module.