map.03 *Cross Flow (TFF) System*

FAST

*A Fully-Automated, True Walk Away*
*and Simplest & Miniaturized Tangential Flow Filtration (TFF)*
*for Sample Concentration and Diafiltration (buffer exchange, desalting)*

## Table of Contents

**Cautions for Operation**

1. Flush ultrafiltration (UF) cartridges prior to use to remove preservative solution and measure clean water flux.
2. Hollow Fiber can't be autoclavable.
3. Membrane water flux may be significantly affected by water quality and temperature as well as wetting and cleaning procedures.
4. Do NOT allow UF cartridges to dry out.
5. Do NOT shock membrane cartridges with rapid temperature changes.
6. Do NOT use tool to do connection.

## 1. Introduction

The MAP.03 Cross-Flow (TFF) Systems is for bench-top scale, the processes ranging is from 2ml to 1L. The ideal is for small volume, R&D scale microfiltration and ultrafiltration due to its miniaturized footprint and versatility. The system allows using both of the flat-sheet TFF cassettes and hollow Fiber.
The systems consist of a Digital Peristaltic Pump, Pump Head, Sample Holder Stand and Fitting Kit. When equipped with optional balance, the system will be automated shut-off controls by permeate weight.

**7 Key Benefit**

1. Simplest and Smallest automatic TFF (Cross Flow) system
2. Easy to use and Save time
3. Wide range of working volume, 1ml-1000ml
4. High recovery rate, up to 95%
5. Efficiency, no residue problem
6. Sterilized & reusable
7. Conveniences, space saving ,

**Application**

- Protein purifications, concentrations, buffer /medium exchange
- Concentrated of Cell, Virus, Bacteria, Alga, protozoa, etc.
- Polysacharides and chitosan concentration and diafiltration
- Liposome, Microparticles, Nanoparticles concentration and buffer exchange
- Desalting (salt removal) and buffer exchange
- Lysate (products of lysis of cells) clarification
- Inclusion body clarification and concentration

## 2. Specifications

- **MAP.03 mini Cross Flow System**

➢ **Output**

- Speed: 1 to 300 rpm (1~200 ml/min)
- Display: LED Backlight

➢ **Input**

- Supply voltage limits: 90 to 260 Vrms @ 50/60 Hz (Universal Input)
- Current, Maximum: 1 A @ 115 Vrms, or 0.5 A @ 230 Vrms

➢ **Construction**

- Dimensions (L × W × H): 160 × 110 × 150 mm
- Enclosure: Stainless Steel

➢ **Environment**

- Temperature, Operating: 0° to 40°C (32° to 104°F)
- Temperature, Storage: –25° to 65°C (–13° to 149°F)
- Humidity (non-condensing): 10% to 90%
- Altitude: Less than 2000 m

- **Permeate Scale #MAP-S-05 & #MAP-S-10**

➢ **Output**

- Range: 0.1 ~5,000gm for MAP-S-05 (0.1 ~10,000gm for MAP-S-10)
- Resolution: 0.1 gm
- Display: LED Backlight

➢ **Input**

- Supply voltage limits: 90 to 260 Vrms @ 50/60 Hz (Universal Input)
- Current, Maximum: 0.5 A @ 115 Vrms, or 0.25 A @ 230 Vrms

➢ **Construction**

- Dimensions (L × W × H): 160 × 110 × 150 mm
- Enclosure: ABS with Stainless Steel plateform

➢ **Environment**

- Temperature, Operating: 0° to 40°C (32° to 104°F)
- Temperature, Storage: –25° to 65°C (–13° to 149°F)
- Humidity (non-condensing): 10% to 90%
- Altitude: Less than 2000 m

## 3. System Parts List

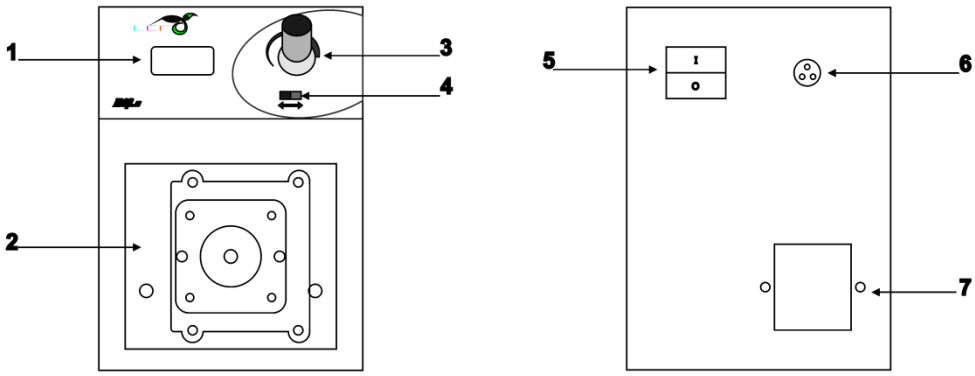
| MAP.03 Cross Flow System | Quantity |
|---|---|
| MAP.03 Driving System | 1 |
| Fitting Kit | 1 |
| Power Supply Cable | 1 |

## 4. Option Item

| Permeate Scale #MAP-S-05 (or #MAP-S-10) | Quantity |
|---|---|
| Permeate Scale | 1 |
| Pump-Scale connecting cable | 1 |
| Transformer | 1 |

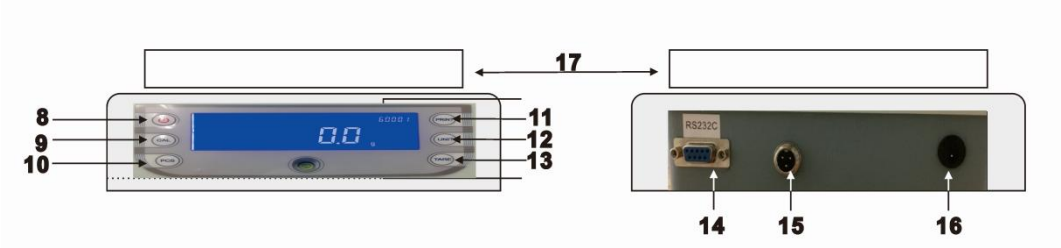
## 5. Panel Controls and Features

### 5.1 MAP.03:



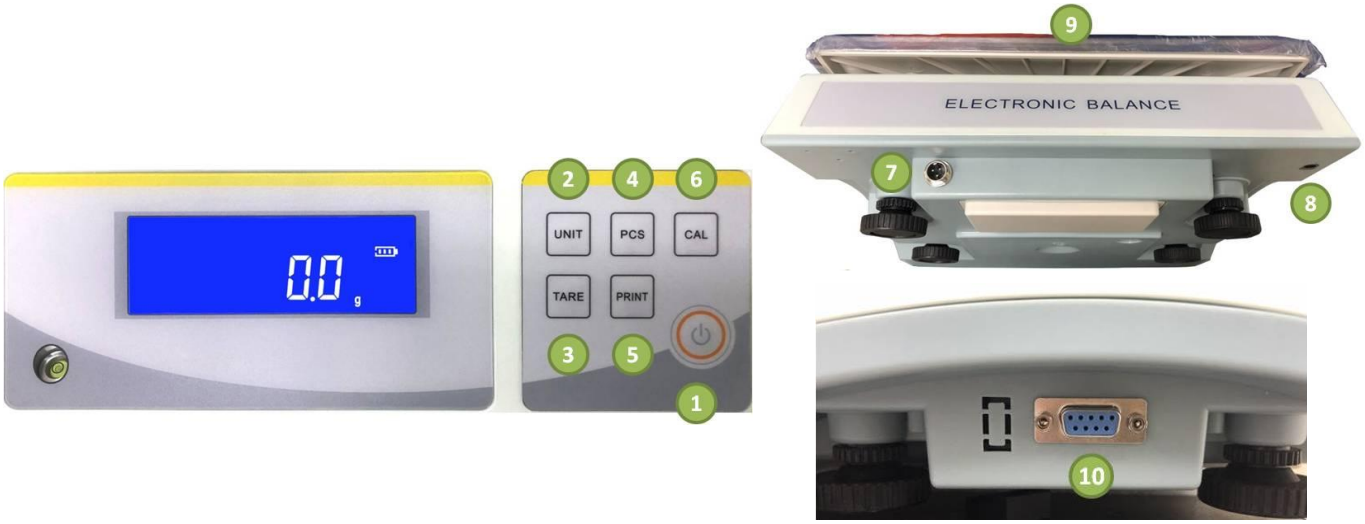

| | | | |
|---|---|---|---|
| 1. | LED display | 5. | Power switch |
| 2. | Pump head | 6. | Scale connecting port |
| 3. | Knob to increase or decrease value | 7. | Power socket |
| 4. | Pump direction switch | | |

### 5.2 Permeate (Filtrate) 6 kg Scale:




| | | | |
|---|---|---|---|
| 8. | Power bottom | 13. | Move Digital or Tare |
| 9. | Back or Calibration | 14. | RS232 |
| 10. | SET | 15. | Pump connecting port |
| 11. | Increase Value | 16. | Power jack |
| 12. | Decrease Value or Unit | 17. | Stainless steel platform |

### 5.3 Permeate (Filtrate) 10 kg Scale:




| | | | |
|---|---|---|---|
| 1. | Power | 6. | CAL |
| 2. | UNIT | 7. | Pump connecting port |
| 3. | TARE | 8. | Power jack |
| 4. | PCS | 9. | Stainless steel platform |
| 5. | PRINT | 10. | VGA connector |

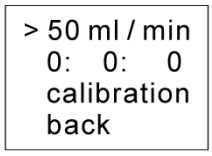
# 6. Setup and Operation

## 6.1 Basic Setup

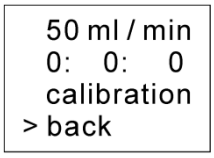
1) Mount the MAP.03 mini Cross Flow system on a flat, horizontal surface. And, connect power cable.
2) If using the Permeate Scale, to connect the Pump-Scale connecting cable.
3) Connect the Hollow Fiber (or Cassette) with tubing to MAP.03 system. (Please refer to 7. Tubing Assembly and pump setup)
4) Switch on MAP.03.

## 6.2 Setting flow rate

1) Press the knob about 3 sec and then release it. Now, it is on the setting mode.
2) To rotate the knob to the flow rate, and click knob.

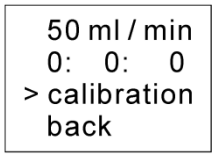



3) To rotate the knob to increase or decrease to require flow rate, and click knob to enter the value.
4) To rotate the knob to move cursor to the “back”, and click knob again to back to main page.

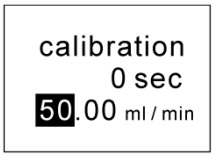



## 6.3 Calibration (If the accuracy flow rate is necessary, please do this function.)

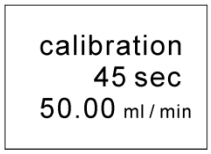
1) Prepare a cylinder. Before do calibration, please priming tubing with water.
2) To rotate the knob to the calibration.




3) Click on the knob to enter the calibration flow function.
4) Rotate the knob to the desired flow rate and click the knob again to start the calibration.

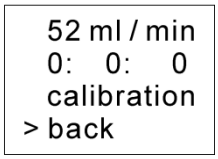


5) Wait for the 60 second, countdown to end




6) To rotate knob to the actual volume of liquid (to measure by a cylinder).


7) To rotate the knob to move cursor to the “back”, and click knob again to back to main page.




Note: If it has larger the difference between the set calibration flow rate and actual flow rate , please repeats calibration, until both are the same.

**6.4 Setting running timer**

1) To rotate knob to move cursor to “hr / min / sec”.
2) And then click knob, the cursor is on “hr”. To rotate knob to increase or decrease the value. And press knob to enter value.
3) To rotate knob to “min”, and then click knob, the cursor is on “min”. To rotate knob to increase or decrease the value. And press knob to enter value.
4) To rotate knob to “sec”, and then click knob, the cursor is on “min”. To rotate knob to increase or decrease the value. And press knob to enter value.
5) To rotate the knob to move cursor to the “back”, and press knob again to back to main page.

   Note:

   a. If timer is set 0:0:0, the pump will be in continue running mode.
   b. If you know the permeate (filtration) rate, you can set timer to stop system automatically. However, we encourage to option the Permeate Scale. The permeate (filtration) rate is not easy to estimation, since it will change by sample viscosity.

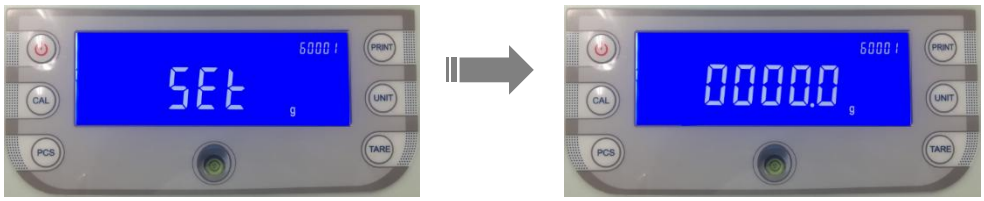
## 6.5 Setting Permeate Scale

The pump will stop automatic after permeate (filtration) weight reach setting point.

### For 6 kg Permeate Scale

1) Hold the power bottom to turn on the scale.

2) Set permeate (filtration) weight:
   a. Press “PCS” for 3 sec, till the LED display “SEt”, and release it.




   b. Click “TARE” to move cursor to digital in ones, tens, hundreds, thousands place etc.
   c. Click “PRINT” and “UNIT” to increase and decrease value for each digital place.
   d. Click “CAL” to finish setting and back to main page.

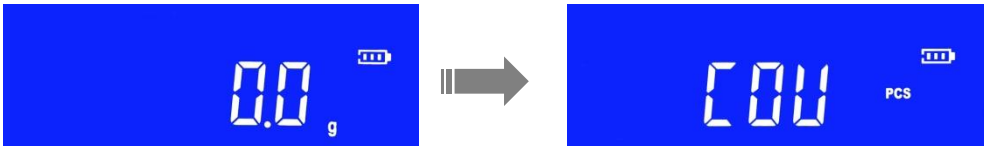

3) Click “TARE” to tare value, before place anything on the scale.

**For 10 kg Permeate Scale**

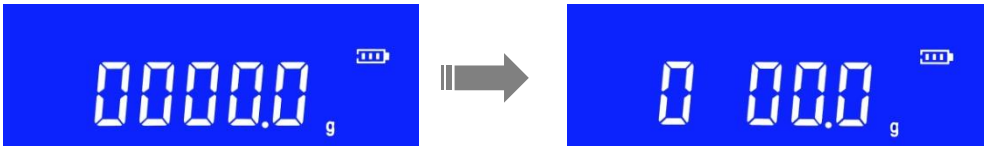
1) Press and hold the power to turn on the scale.

2) Set permeate (filtration) weight:

   e. Press “PCS” for 3 sec until the LED display “COU”, and release it.

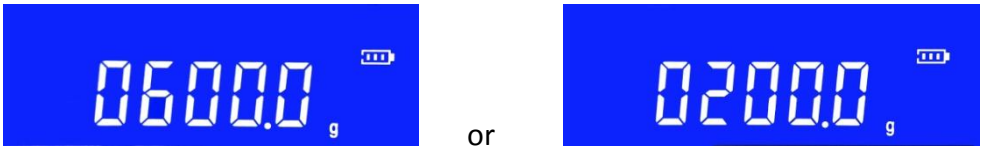



   f. Click “TARE” to move cursor at one, ten, hundred or thousand place etc.



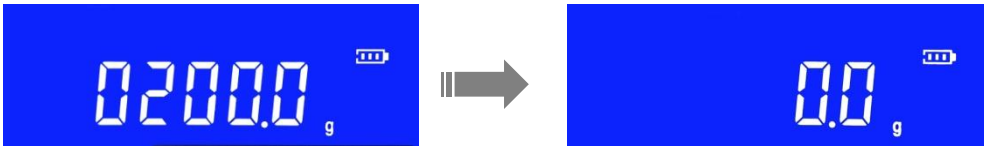

(ex: Cursor at hundred place)

   g. Click “PRINT” or “UNIT” to increase or decrease value for each digital place.




(PRINT to increase) (UNIT to decrease)

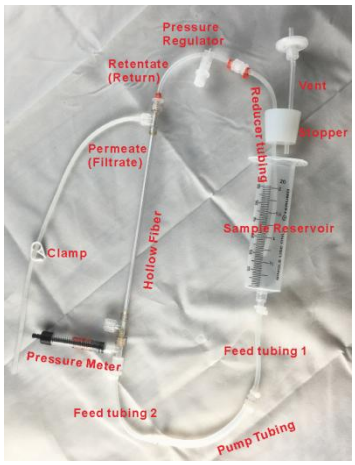
   h. Click “CAL” to finish setting and back to main page.




3) Click “TARE” to tare value to zero.

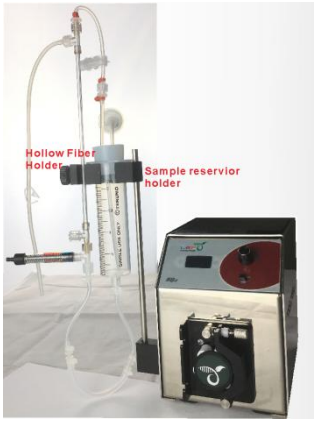
# 7. Tubing Assembly and pump setup

## 7.1 Tubing Assembly Setup




## 7.2 Pump with Tubing Assembly Setup

1) Put the Sample Reservoir and Hollow Fiber to the holders.




2) Click the nut for open pump clamp

3) Fit the pump tubing to the pump head, and then clamp the pump clamp.

4) Put the permeate (filtrate) tubing to the Filtrate Bottle


5) To setting flow rate as we advise (please refer to 10. Hollow Fiber Information), and then start the pump. To check pressure meter always, and adjust it by pressure regulator. Please note, the pressure should keep lower than the Red area. We advise you keep the pressure within on the orange area.

6) To click knob to manually stop the system.

**7.3 Pump ; Scale with Tubing Assembly setup**

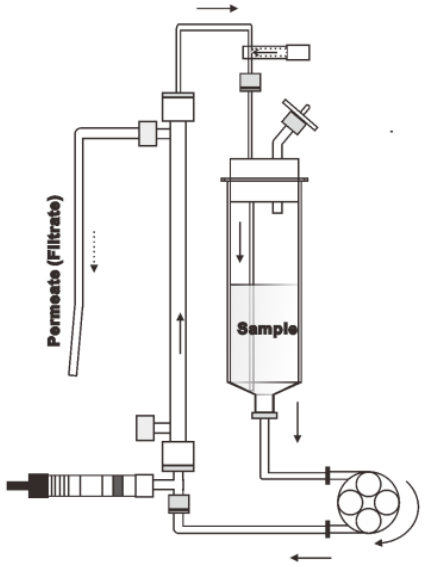
1) To setup pump and tubing assembly, please follow up upon step 1) ~ 3)
2) To move the Permeate (Filtrate) bottle to the Permeate Scale. To set the Permeate (Filtrate) weight (please refer to 6.5 Permeate Scale setup procedure)
3) Press “TARE”.
4) To setting flow rate, and then start the pump. To check pressure meter always, and adjust it by pressure regulator. Please note, the pressure should keep lower than the Red area. We advise you keep the pressure within on the orange area.
5) When permeate (filtrate) bottle reach the setting weight, the system will stop automatically.

**7.4 Collect the Retentate and Permeate Samples:**

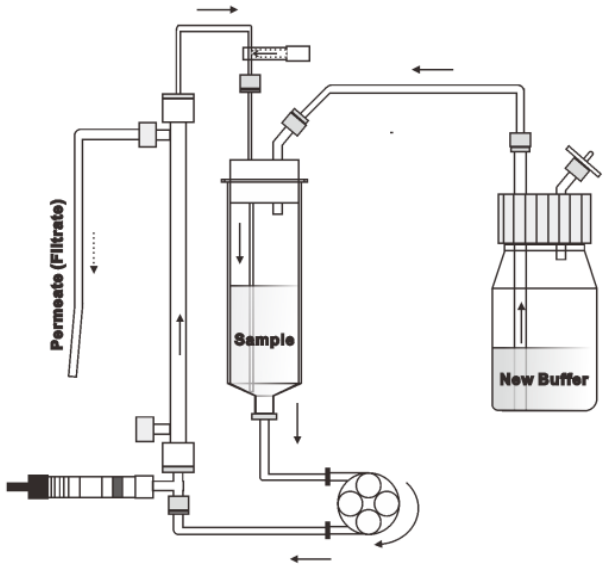
1) Clamp Permeate (Filtrate) tube, and connect a syringe to lower permeate (filtrate) port.
2) Release Permeate (Filtrate) tube clamp, and use syringe to collect permeate (filter) completely.
3) Remove out the stopper
4) Reduce flow rate under 30ml/min, and change pump direction.
5) Start collect sample from hollow fiber back to the reservoir. The flow rate must be as slow as possible, to avoid the sample foaming.
6) To use a pipette to collect sample out.

**7.5 Different connecting types for different application:**

1) Concentration Process for sample volume under 50 ml

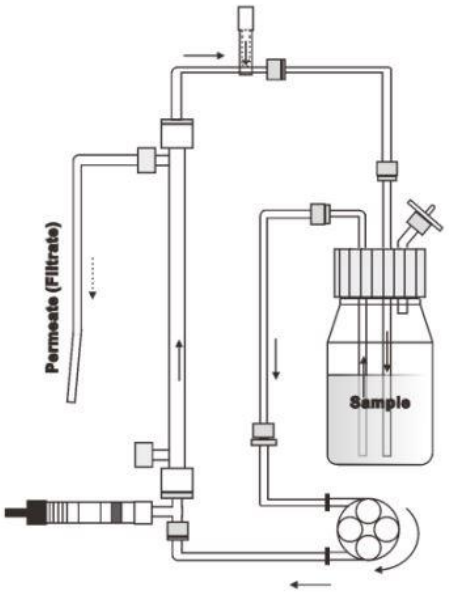



2) Diafiltration (Buffer Exchange) Process for sample volume under 50 ml

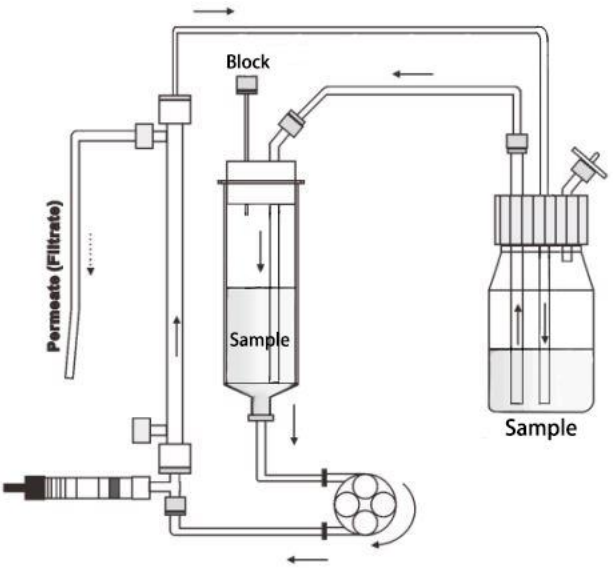


3) Concentration Process for sample volume larger than 50 ml

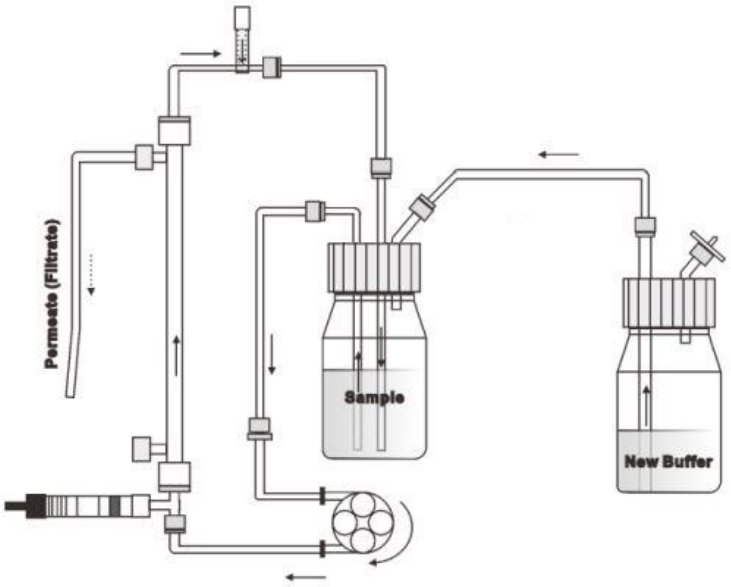
I. 1 bottle




II. 2 bottles




4) Diafiltration (Buffer Exchange) Process for sample volume larger than 50 ml



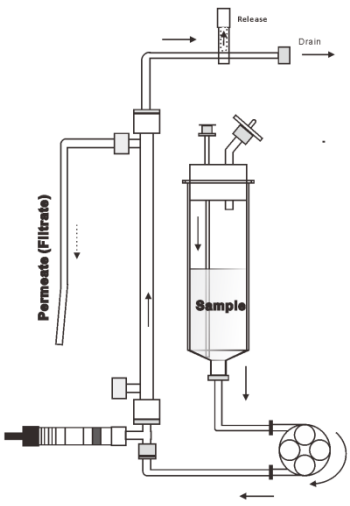
## 8. Clean Procedures

### 8.1 Cleaning Procedures Recommend

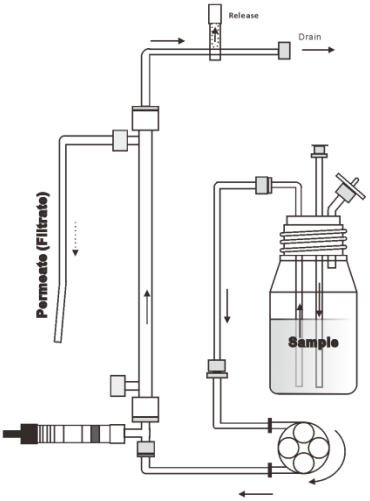
| Process | Foulants | Cleaning Procedures i |
|---|---|---|
| Mammalian Cell Culture | Cell Debris | 1. Flush with clean water, buffer or saline at 50 °C<br>2. Circulate 0.5N NaOH at 50 °C, 1 hr.<br>3. Flush with clean water. |
| Bacterial Cell Whole Broths | Proteins, Cell Debris, Poly saccharides, Lipids, Antifoams | 1. Flush with clean water, buffer or saline at 50 °C.<br>2. Circulate 0.5N NaOH at 50 °C, 1 hr.<br>3. Flush with clean water.<br>4. Circulate NaOCl* at 50 °C, pH 10-11, 1 hr.<br>5. Flush with clean water. |
| Blood & Serum Products, Enzymes, Vaccines, Protein Products | Proteins, Lipoproteins, Lipids | |
| Juice and Beverage Clarification | Protein, Pectin, Colloids, Tannins, Polyphenolics | |
| Bacterial Cell Lysates | Proteins, Cell Debris | 1. Flush with clean water, buffer or saline at 50 °C.<br>2. Circulate 0.5N NaOH at 50 °C, 1 hr.<br>3. Flush with clean water.<br>4. Flush with H3PO4 at 50 °C, pH 4, 1 hr.<br>5. Flush with clean water. |
| Dairy | Protein, Insoluble Calcium Complexes | 1. Flush with clean water.<br>2. Circulate H3PO4 at 50 °C, pH 3.5-4 for 20 mins.<br>3. Flush with clean water.<br>4. Circulate 0.5N NaOH at 50 °C for 20 minutes.<br>5. Flush with clean water.<br>6. Circulate NaOCl* at 50 °C, pH 10-11 for 1 hour. Monitor and maintain chlorine lev el.<br>7. Flush with clean water. |

**Flush**

A. To flush clean water or buffer about 10 minutes. Typical solution consumption is 3 to 4 times the system hold-up volume.
B. Drain solution.




For process volume under 50 ml



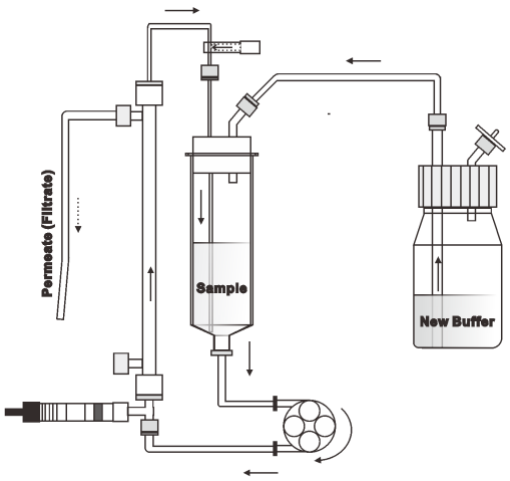

For process volume up to 50 ml

**Clean**

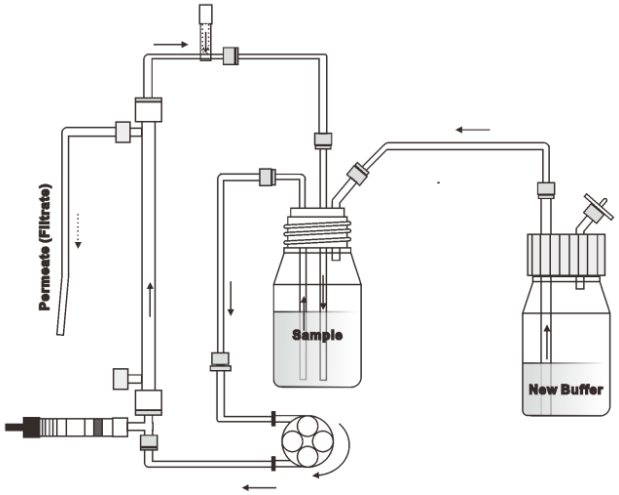
C. Recirculate cleaning solution in "total recycle" at 4000 to 8000 sec-1 shear rate for 30 to 60 minutes with 5 psig (0.3 barg) outlet pressure. Typical solution consumption is 3 to 4 times the system hold-up volume.
D. Drain solution.

**Rinse**

E. Recirculate clean water or buffer in "total recycle" for 5 minutes at 8000 to 16000 sec-1 shear rate, with 5 psig (0.3 barg) outlet pressure. Typical solution consumption is 2 times the system hold-up volume.
F. Drain solution.
G. Repeat rinse water recirculation/drain steps "E" & "F" two more times.
H. Rinse both retentate and permeate sides with clean water/buffer, controlling the permeate rate at 0.1 liters/minute/sq. ft. of membrane area. The retentate flow rate should be nominally 5 - 10% of the permeate flow rate.
I. It is preferred that the cartridge be oriented vertically and permeate be removed from the upper permeate port.
J. Continue to rinse for 60 minutes. [Note: 60 minutes is a nominal time frame. To conserve water/buffer, time may be reduced to 30 minutes.]




For process volume under 50 ml




For process volume up to 50 ml

Note:

1) See Shear Rate, please refer 10. Hollow Fiber Information.
2) Total Recycle = Return both retentate and permeate streams to the feed reservoir.

## 8.2 Sanitization

For sanitization, thoroughly clean and rinse the membrane cartridges, then use any of the following:

1. Up to 0.5 N sodium hydroxide. Circulate 30 to 60 minutes.
2. Up to 100 ppm* sodium hypochlorite. If properly cleaned, 10 ppm should be sufficient. Circulate 30 to 60 minutes.

## 8.3 Depyrogenation

For depyrogenation, thoroughly clean, sanitize and rinse the membrane cartridges, then recirculate either of the following for 30 to 60 minutes at 30° to 50 °C. Then thoroughly drain & flush with non-pyrogenic water.

1. 100 ppm sodium hypochlorite, pH 10 to 11.
2. 0.1N to 0.5 N sodium hydroxide, pH 13.

## 8.4 Storage

Ultrafiltration cartridges must be stored wet or reglycerized. Before storage the cartridges should be thoroughly flushed, cleaned and rinsed with clean water. For short-term storage, up to two weeks, cartridges need only be water-wet. For storage up to 1 month, cartridges may be filled with a storage solution and sealed at all endfittings and permeate ports, or submerged in a storage bath.

Acceptable storage solutions are:

1. Water with 5 to 10 ppm active chlorine (10 to 20 ppm sodium hypochlorite). Monitor levels weekly.
2. 0.1 N sodium hydroxide.
3. Up to 3% formalin.
4. 30% ethanol in water.
5. Up to 1% sodium azide.

For storage of longer than 1 month, check periodically to be certain that the membranes remain wetted. Prior to reuse it is recommended that the cartridge be rinsed with a 100 ppm sodium hypochlorite solution. Thoroughly rinse all storage solution prior to reuse.

Microfiltration cartridges may be stored dry, after cleaning. It is advisable to clean and sanitize the microfiltration cartridges prior to reuse. If necessary, to fully wet the membranes after extended storage, expose the membranes [inside and outside] to 70% alcohol for one hour. Drain, wet with water and rinse.

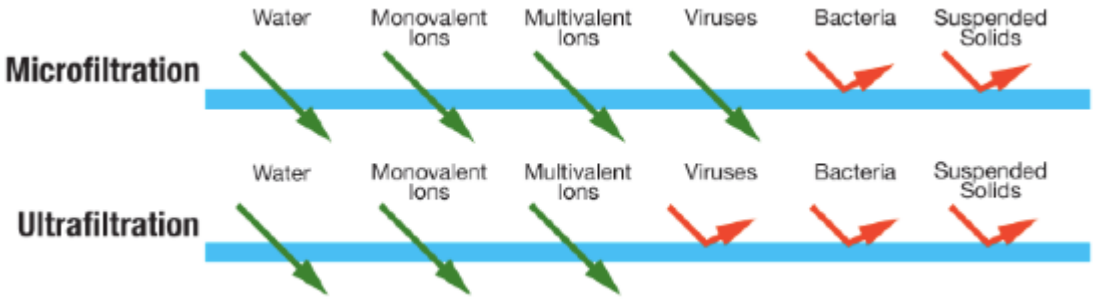
# 9. Basic Concepts of Tangential Flow Filtration

## 9.1 Introduction

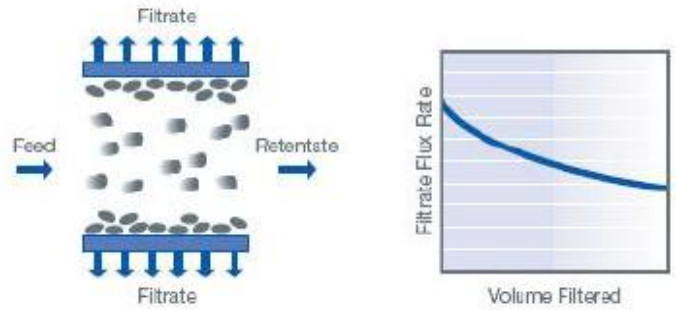
Membrane filtration is a separation technique widely used in the life science laboratory. Depending on membrane porosity, it can be classified as a microfiltration or ultrafiltration process.

Microfiltration membranes, with pore sizes typically between 0.1 µm and 10 µm, are generally used for clarification, sterilization, and removal of micro-particulates or for cell harvesting.

Ultrafiltration membranes, with much smaller pore sizes between 0.001 and 0.1 µm, are used for concentrating and desalting dissolved molecules (proteins, peptides, nucleic acids, carbohydrates, and other biomolecules), exchanging buffers, and gross fractionation. Ultrafiltration membranes are typically classified by molecular weight cutoff (MWCO) rather than pore size.




The Tangential Flow Filtration (TFF), also known as crossflow filtration, where the feed stream passes parallel to the membrane face as one portion passes through the membrane (permeate) while the retentate is recirculated back to the feed reservoir.



**Glossary of Terms**

Cross Flow: Sweeping action created by fluid flow across the membrane (also called tangential flow).

Feed Stream: Bulk solution to be processed via ultrafiltration or microfiltration (also called process solution).

Retentate: Solution containing species retained by the membrane (also called concentrate).

Permeate: Solution containing solvent and solutes passing through the membrane (also called filtrate)

## 9.2 Membrane Pore Size Selection

The molecular weight cutoff (MWCO) of a membrane is defined by its ability to retain a given percent of a molecule in solution (typically 90% retention).

To retain a product, select a membrane with a MWCO that is 3 to 6 times lower than the molecular weight of the target protein. For fractionation, select a membrane MWCO that is lower than the molecular weight of the molecule to be retained but higher than the molecular weight of the molecule you are trying to pass.

## 9.3 Micorfiltration and Ultrafiltration

With a pore size of approximately 0.1-10 µm and a molecular weight cut-off range of 0.2-0.08µ, microfiltration membranes are able to remove larger contaminants and even some viruses from a feed stream. In terms of solute rejection, MF is typically measured via gas permeation (i.e. using bubble points or gas-liquid porosimetry). With these larger pore sizes, MF is commonly used in applications such as beverage sterilization, wastewater treatment, and oil separation.

Ultrafiltration membranes have a slightly smaller pore size range of 0.001-0.1µm, and a molecular weight cut-off range of 1k-500k Da. They are higher overall retention rate when compared to MF membranes, which allows for further rejection of all viruses, macromolecules, and proteins. Regarding solute rejection, UF can be measured by passing molecules with a known molecular weight through the membrane (i.e. using markers such as polyethylene glycol or dextran). This type of filtration is most applicable for separation in the food and dairy industries, biotech, pharmaceutical, as well as in the automotive industry for cathodic paint recovery.

### 9.4 Concentration

Concentration is the reduction of the initial sample volume to a lower, final sample volume. The opposite would be a dilution—where the initial sample volume is lower than the final sample volume through the addition of buffer or other medium. If the process volume is 10L and needs to be concentrated to 10X, then the final sample volume will be 1L. By removing 1-5% of each sweep of recirculated process volume, SpectrumLabs.com's HF TFF modules will gradually reduce the initial sample volume's weight. When using Feed and Permeate Scales, this weight loss is communicated to the TFF system so that the TFF system knows when the process volume has been reduced to its desired concentration factor. As the weight decreases on the Feed Scale and increases on the Permeate Scale, the TFF system knows that the process volume is being concentrated. If the weight stays constant on the Feed Scale while increasing on the Permeate Scale, the TFF system knows that the volume is undergoing a fed-batch concentration.

### 9.5 Diafiltration

Diafiltration is the washing of cells, cell debris, virus, precipates, proteins, and other materials. For instance, this is often done as an efficient method of buffer exchange, for instance. Diafiltration is measured by how many washes the process volume has undergone. If the process volume is 10L and needs to be washed 5 times, then 50L of diafiltration buffer must wash through the process volume.

The TFF system's Diafiltration function relies upon feedback from both the Feed and Permeate Scales. The TFF system's will add buffer to maintain the concentrated weight on the Feed scale. Once a set amount of weight is reached on the Permeate Scale—which would indicate that a set amount of Diafiltration Volumes have washed through the process volume—the TFF system will know that the process volume has been washed a desired number of times.

## 10. Hollow Fiber Information

**Application :** Material : mPES ; Fiber ID = 0.5 mm

- Protein purifications, concentrations, buffer /medium exchange
- Polysacharides and chitosan concentration and diafiltration
- Liposome, Microparticles, Nanoparticles concentration and buffer exchange
- Desalting (salt removal) and buffer exchange

| For under 50 ml | For under 100 ml | For under 500 ml | For under 1,000 ml | MWCO |
|---|---|---|---|---|
| SA= 20 $cm^2$; | **SA= 40 $cm^2$;** | **SA= 115 $cm^2$;** | **SA= 235 $cm^2$;** | |
| Under 4000 sec-1 shear rate = under 18 ml/min<br>4~6000 sec-1 shear rate = 18~27 ml/min<br>6~8000 sec-1 shear rate = 27~35 ml/min<br>Up to 8000 sec-1 shear rate =35 ml/min | | Under 4000 sec-1 shear rate = under 100 ml/min<br>4~6000 sec-1 shear rate = 100~160 ml/min<br>6~8000 sec-1 shear rate = 160~220 ml/min<br>Up to 8000 sec-1 shear rate =220 ml/min | | |
| C02-E001-05-N | C04-E001-05-N | D02-E001-05-N | D04-E001-05-N | 1 kD |
| C02-E003-05-N | C04-E003-05-N | D02-E003-05-N | D04-E003-05-N | 3 kD |
| C02-E005-05-N | C04-E005-05-N | D02-E005-05-N | D04-E005-05-N | 5 kD |
| C02-E010-05-N | C04-E010-05-N | D02-E010-05-N | D04-E010-05-N | 10 kD |
| C02-E030-05-N | C04-E030-05-N | D02-E030-05-N | D04-E030-05-N | 30 kD |
| C02-E050-05-N | C04-E050-05-N | D02-E050-05-N | D04-E050-05-N | 50 kD |
| C02-E070-05-N | C04-E070-05-N | D02-E070-05-N | D04-E070-05-N | 70 kD |
| C02-E100-05-N | C04-E100-05-N | D02-E100-05-N | D04-E100-05-N | 100 kD |
| C02-E300-05-N | C04-E300-05-N | D02-E300-05-N | D04-E300-05-N | 300 kD |
| C02-E500-05-N | C04-E500-05-N | D02-E500-05-N | D04-E500-05-N | 500 kD |
| C02-E750-05-N | C04-E750-05-N | D02-E750-05-N | D04-E750-05-N | 750 kD |

**Application :** Material : mPES; Fiber ID = 1.0 mm

- Concentrated of Cell, Virus, Bacteria, Alga, protozoa, etc.
- Lysate (products of lysis of cells) clarification
- Inclusion body clarification and concentration

| For under 50 ml | For under 100 ml | MWCO |
|---|---|---|
| SA= 13 $cm^2$; | **SA= 26 $cm^2$;** | |
| Under 4000 sec-1 shear rate = under 50 ml/min<br>4~6000 sec-1 shear rate = 50~70 ml/min<br>6~8000 sec-1 shear rate = 70~90 ml/min<br>Up to 8000 sec-1 shear rate =90 ml/min | | |
| C02-E500-10-N | C04-E500-10-N | 500 kD |
| C02-E750-10-N | C04-E750-10-N | 750 kD |