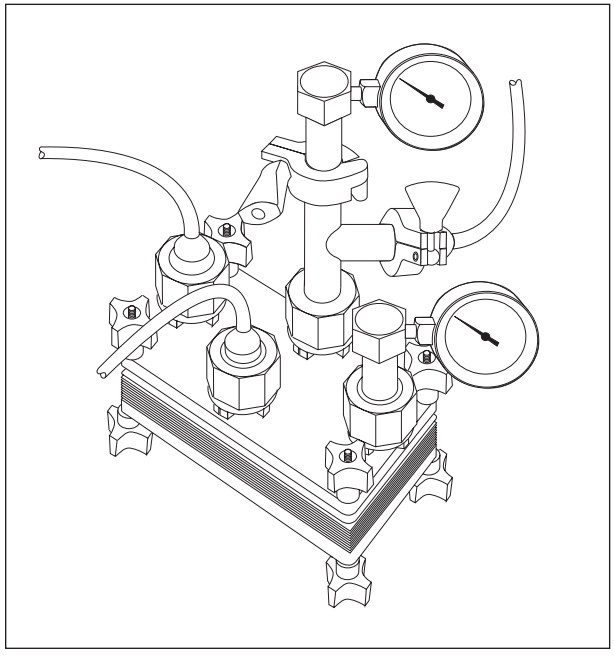
Viresolve®

Virus Removal Module

User Guide



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User Guide

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Introduction

Overview

This chapter provides information on:

- Description of the Viresolve® Virus Removal Module
- Contents of the Package
- Required Accessories
- Primer on Tangential Flow Filtration

Description of the Viresolve® Virus Removal Module

The Viresolve® Virus Removal Module is a self-contained, single-use disposable module designed to remove viruses from protein solutions. Viresolve modules are used in tangential flow filtration mode with two peristaltic pumps, for superior transmembrane pressure control.

Each module is supplied with a medical-grade silicone tubing set and gaskets, for easy connection.

In addition to the two pumps, a manifold and other accessories are necessary to install the module, but are not included in the Viresolve module package. These components are described below, in Required Accessories.

Viresolve modules are available with either of two membrane types. A Viresolve/70 module provides membrane that will pass proteins of 70,000 molecular weight (MW) and smaller. A Viresolve/180 module provides membrane that will pass proteins of 180,000 MW and smaller.

In addition, Viresolve modules are available in the following sizes:

Module	**Nominal Membrane Area**	
one-stack	150 cm^2	(1/6 ft^2)
two-stack	0.03 m^2	(1/3 ft^2)
six-stack	0.1 m^2	(1 ft^2)

The catalogue numbers for the available Viresolve modules are listed at the end of this User Guide, under Product Ordering Information, in the chapter on Reference Information.

If you are new to tangential flow filtration (TFF), you may also want to read the TFF overview provided at the end of this chapter, introducing TFF terminology and principles.

Contents of the Package

Before installing the Viresolve Virus Removal Module, please check that the package contents are complete, and that you have received the correct module size and membrane type for your application. Do not unwrap any items until you have verified the contents of the package.

The package should contain the following items:

- One Viresolve module
- Tubing, individually packaged, consisting of one feed/buffer line, one retentate line, and one permeate line

  The three packages of tubing are identified in Chapter 2, Assembly.
- Four gaskets (for the module)
- One gasket (for the minicap)
- One User Guide
- One Certificate of Quality

Each Viresolve module is labeled, and its label should be visible through the packaging.

The label identifies the module by membrane type — as either a Viresolve/70 or Viresolve/180 module — and indicates the nominal membrane area of the module. (The options available are described in the overview at the beginning of this chapter.)

Please contact Millipore if your package is incomplete or if you have not received the required module size and membrane type. Do not unwrap any items.

If your package is complete and correct, please proceed to the next section for an introduction to tangential flow filtration, or to Chapter 2, Assembly, for instructions on how to install the module.

Required Accessories

The accessories described in this section are **not** included with the Viresolve module.

The catalogue numbers for these accessories are repeated at the end of this User Guide, under Product Ordering Information, in Chapter 6, Reference Information.

Viresolve Manifold Kit

A manifold is necessary to install the module, but is not included in the Viresolve module package. The Viresolve Manifold Kit is available from Millipore as catalogue number VMSP 000 02.

The manifold kit contains:

- One each, upper and lower manifold plates
- Four sets of connecting hardware (thumbscrews and wingnuts)
- Four sets of true union connectors
- One minicap

Peristaltic Pumps

Two peristaltic pumps are required to operate the Viresolve system. Millipore offers an Easy-Load peristaltic pump head capable of 1,680 mL/min at 600 RPM, catalogue number XX80 EL0 05.

The pump drive is not included with the pump head and must be ordered separately. It is a variable speed, 115 V/ 60 Hz, 1.5 A, 60 – 600 RPM drive, catalogue number XX82 00 115. Outside North America, please contact your local Millipore representative concerning equipment suitable for use with your electrical supply.

Pressure Dispensing Vessel

In order to properly wet out the Viresolve module in the autoclaving procedure, and for accurate integrity testing of the Viresolve system, a Pressure Dispensing Vessel with a minimum capacity of 2 liters is recommended. A 1 gallon (3.78 L) vessel is available from Millipore, with catalogue number XX67 00P 01.

Required Accessories, continued

Pressure Gauges

For accurate monitoring of the process system, pressure gauges should be placed in-line. Two gauges are recommended, one for the feed port and one for the permeate port. An additional gauge can also be used on the retentate. The gauge is an autoclavable analog gauge with a fractional sanitary fitting which can be ordered with catalogue number B26524 (1 per package).

Gauge Adapter Kit

The gauges can be attached in-line with the ports using catalogue number XX42 PM0 01, which contains two stainless steel tees, four silicone fractional sanitary gaskets, and four stainless steel fractional sanitary fitting clamps.

The CorrTest™ Integrity Test Kit

To completely validate virus removal by the Viresolve module within a specific process run, it is necessary to have some means of verifying pre- and post-processing membrane integrity. Millipore developed a patented liquid/ liquid integrity test, CorrTest, to meet this need. The test is not included as part of the Viresolve module package but can be ordered separately using catalogue number VMVC T06 S3. Please call you local Millipore representative for assistance with this product.

Primer on Tangential Flow Filtration

There are two types of filtration used in separations: normal (otherwise known as dead-ended) flow and tangential flow.

In normal flow filtration, all flow is directed through the membrane, with retained material building up on the surface of the filter.

In tangential flow filtration (TFF), flow is directed across the membrane surface in a "sweeping" motion, as illustrated in Figure 1. The sweeping action of tangential flow filtration helps keep material retained by the membrane from creating a layer on the filter surface, a condition known as "concentration polarization."

Tangential flow filtration is used to concentrate and/or desalt solutes retained by the membrane (retentate) or to collect material passing through the membrane (permeate). Materials smaller than the pore size or nominal molecular weight limit (NMWL) are able to pass through the membrane and may be depyrogenated, clarified, or separated from higher molecular weight or larger species. Materials larger than the pore size or molecular weight cutoff are either "rejected" or retained by the membrane and may be concentrated, and washed or separated from lower molecular weight species.

Retention and/or passage of molecules for any tangential flow filter are affected by the purity and concentration of the protein, solution chemistry, pH, conductivity, temperature, tangential flow rate and permeate flow rate.

The Viresolve system operates in a TFF mode, employing two pumps. The two-pump system allows for high-resolution separation and control of the concentration polarization at the membrane surface.

Primer on Tangential Flow Filtration, continued

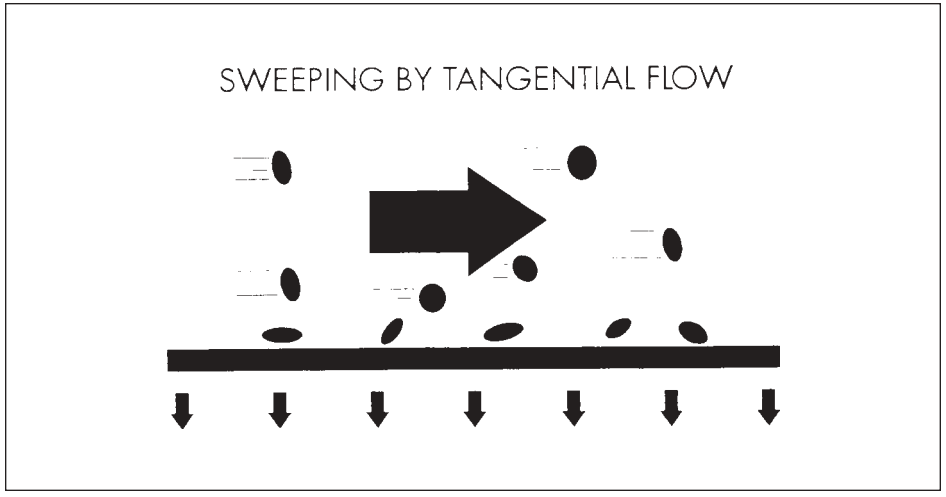

Figure 1. Sweeping Motion of Tangential Flow Filtration

2

Assembly

Overview

This chapter provides information on:

- Assembly of the Viresolve manifold with the module
- Installation of the in-line pressure gauges and tubing
- Installation of the minicap (optional alternative)

Materials and Equipment

To assemble the Viresolve Virus Removal System, the following items are required, in addition to the materials provided with the Viresolve module:

- Viresolve Manifold Assembly Kit
- Two peristaltic pumps
- Two gauges
- Tees, clamps and gaskets (provided in the Gauge Adapter Kit)

These items are described in detail in the previous chapter, under Required Accessories.

Standard Assembly Procedure

The standard assembly procedure consists of four operations, which are described on the following pages:

- Installing split nuts onto the manifold fittings
- Installing the module in the manifold
- Attaching the pressure gauges to the manifold/module assembly
- Attaching the tubing to the manifold/module assembly

Installing Split Nuts onto the Manifold Fittings

Install the split nut, “feet” down, around each sanitary fitting as shown in Figure 2. Keep the seam of the split nut parallel with the outer edge of the upper manifold plate. You will hear a “click” as the two pieces join.

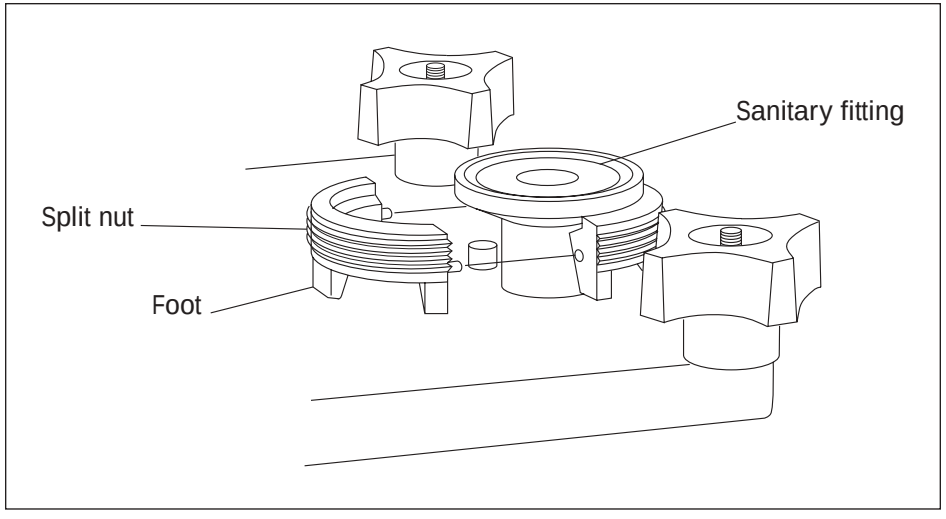

Figure 2. Installation of Split Nut on Manifold Fitting

Installing the Module in the Manifold

Refer to Figure 3 for placement of system components in the manifold/module assembly.

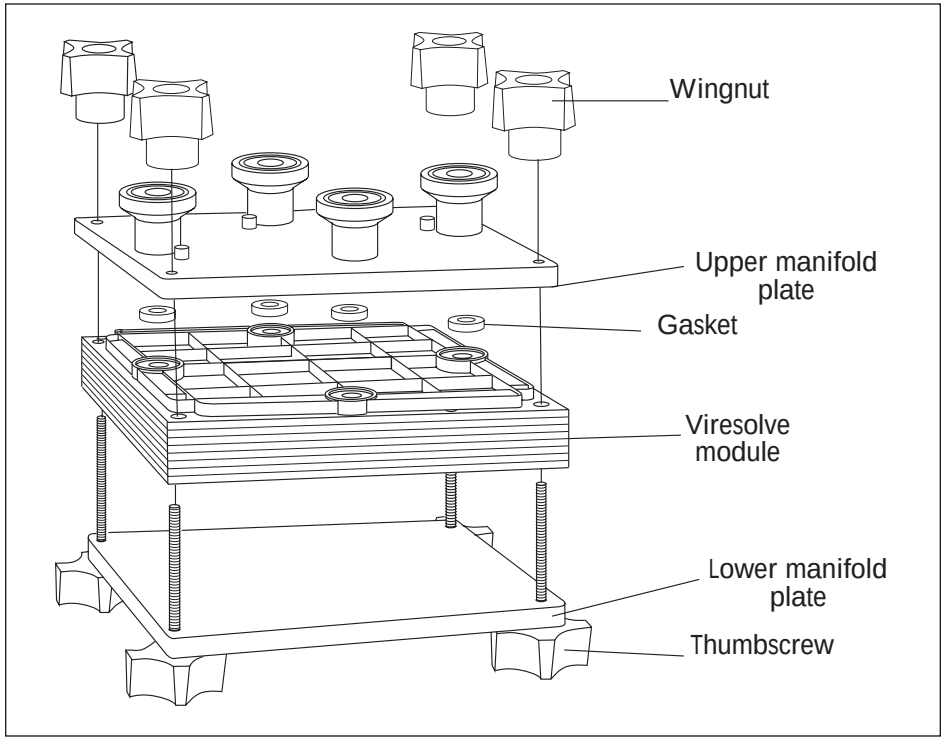

Figure 3. Schematic of Viresolve Manifold Assembly

Installing the Module in the Manifold, continued

1. Insert the thumbscrews through the corner holes of the lower plate, from the bottom up. (The lower plate is smooth, without fittings. The bottom of the lower plate is marked with the Millipore logo.)
2. Slide the module, fitting side up and smooth side down, over the four thumbscrews and onto the lower manifold plate.

   NOTE: Modules are **not** stackable. The manifold assembly holds only one module at a time.
3. Place a silicone gasket in the groove of each fitting on the module. Be sure that each gasket is evenly seated.
4. Slide the upper manifold plate, fitting side up, over the thumbscrews, so that it fits flush on the module. Be sure that the gaskets remain seated.
5. Thread a wingnut onto each of the four thumbscrews until snug.
6. Tighten each of the four wingnuts with an additional half turn.

Installing the Module in the Manifold, continued

The assembly is now ready for use in your application, as shown in Figure 4. Please note the location of the permeate, feed and retentate fittings, which are labeled on the upper manifold plate.

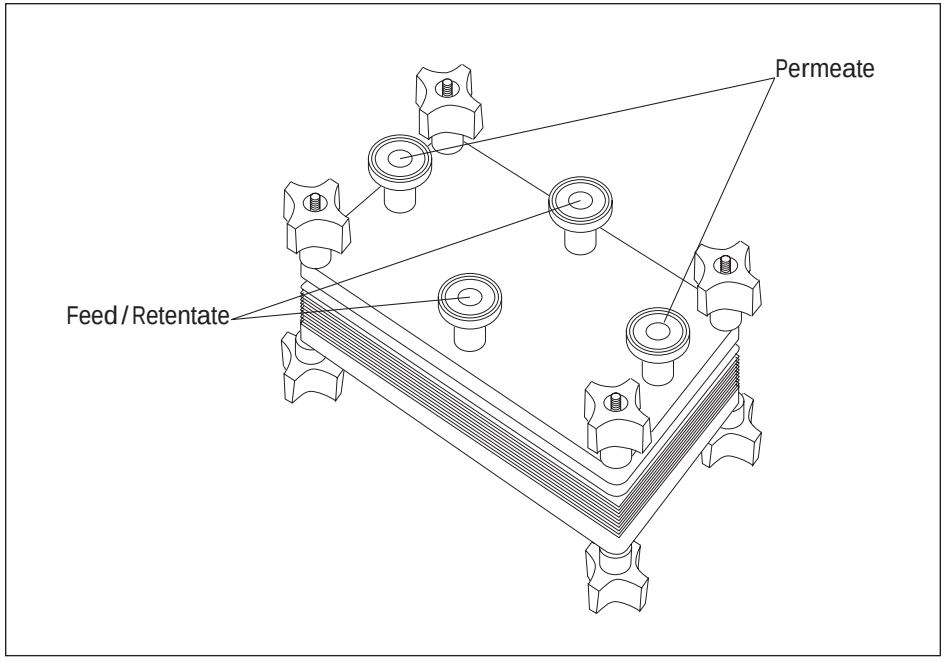

Figure 4. Module Assembled in Manifold

NOTE: The feed and retentate fittings are interchangeable. Only one permeate fitting is used; the second fitting may be either capped or fitted with a gauge.

The remaining procedures assume that fittings have been designated for feed, permeate or retentate.

Attaching the Pressure Gauges to the Manifold / Module Assembly

First, a tee is connected to one of the feed/retentate fittings on the manifold, using a true union connector. (This will be the feed port.) A pressure gauge is then attached to the tee, to monitor feed pressure.

A second pressure gauge is attached to one of the permeate fittings, using a true union connector. This gauge will monitor permeate pressure.

The true-union connector, shown in Figure 5, has three pieces:

- split nut
- ferrule
- union nut

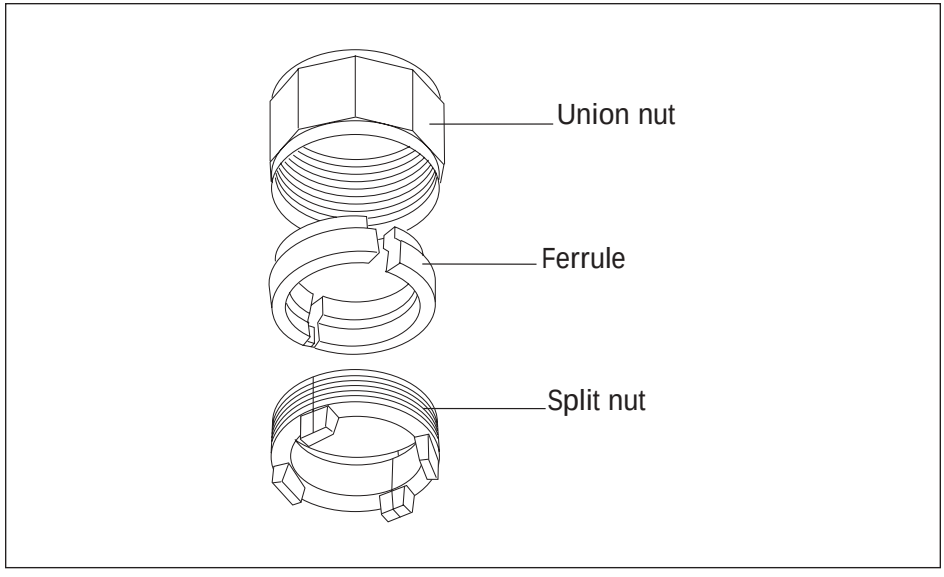

Figure 5. Components of a True-Union Connector

Attaching the Pressure Gauges to the Manifold/Module Assembly, continued

Split nuts have been installed already on the sanitary fittings on the upper manifold plate. On both the tee and the permeate pressure gauge, a ferrule and union nut will be installed, forming a fitting, which will be screwed onto the split nut, to make a sanitary connection between the tee or gauge and the manifold.

1. Install a union nut and ferrule on one of the sanitary fitting ends of a tee.
   a. Slide the union nut and the ferrule over the fitting end of the tee in the sequence and orientation shown in Figure 6.

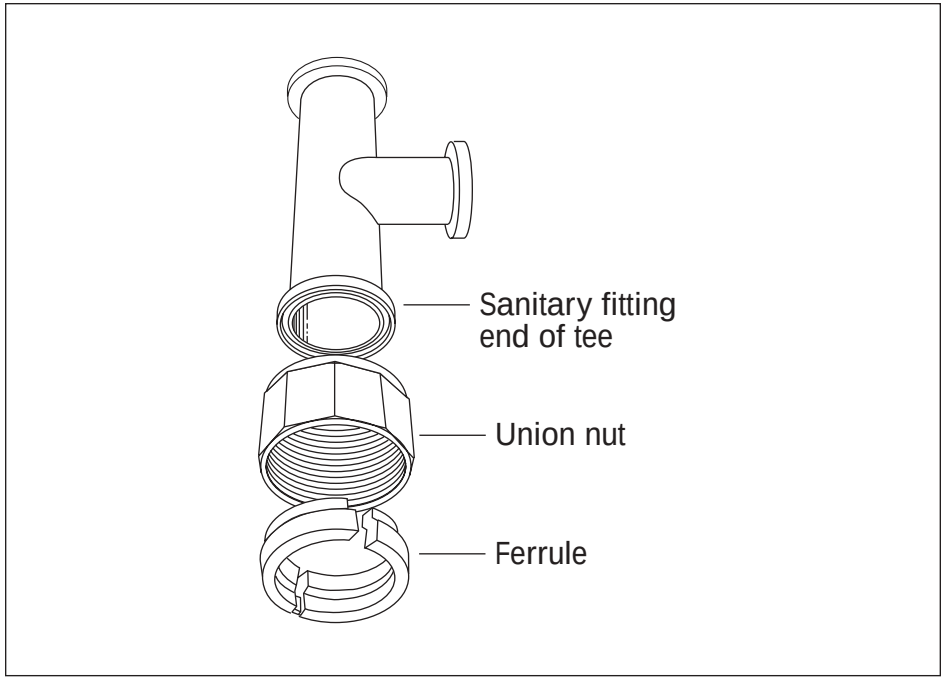

Figure 6. Sequence of Ferrule and Union Nut on Tee

Attaching the Pressure Gauges to the Manifold / Module Assembly, continued

b. Compress the ferrule, so that it slides into the union nut.

c. Push the union nut downward over the sanitary fitting end of the tee (as shown in Figure 7) until it forms a snug cap. You will hear it "click" into place.

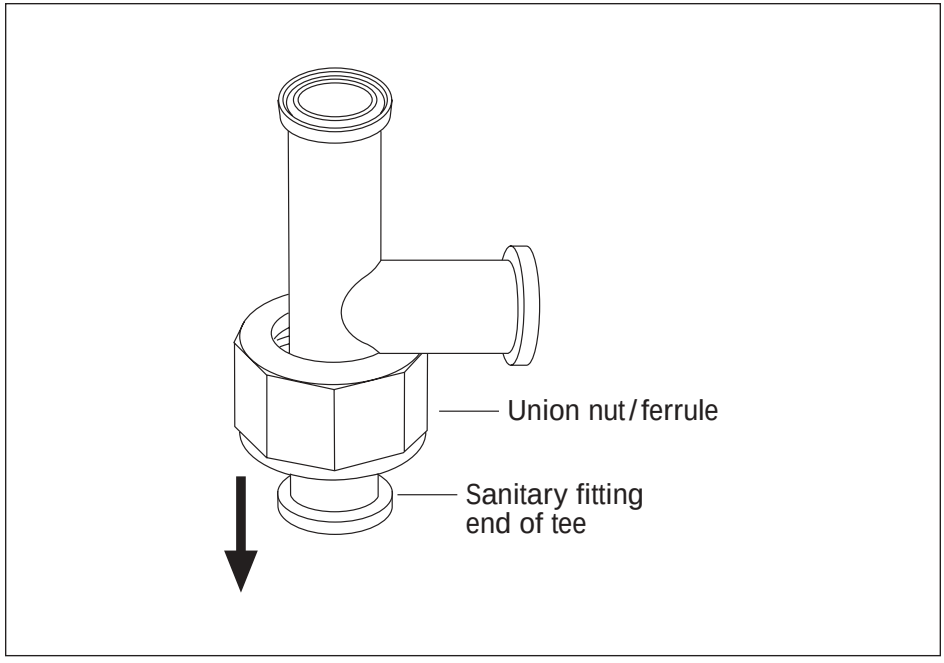

Figure 7. Union Nut and Ferrule on Tee

2. Install the tee on the manifold in the selected feed position (see Figure 4).

   a. Place the tee, with the union nut and ferrule, over the fitting.

   b. Screw the combined union nut and ferrule onto the split nut until finger-tight.

Attaching the Pressure Gauges to the Manifold/Module Assembly, continued

3. Secure a pressure gauge to the tee. (The sequence and orientation of the components are shown in Figure 8.)

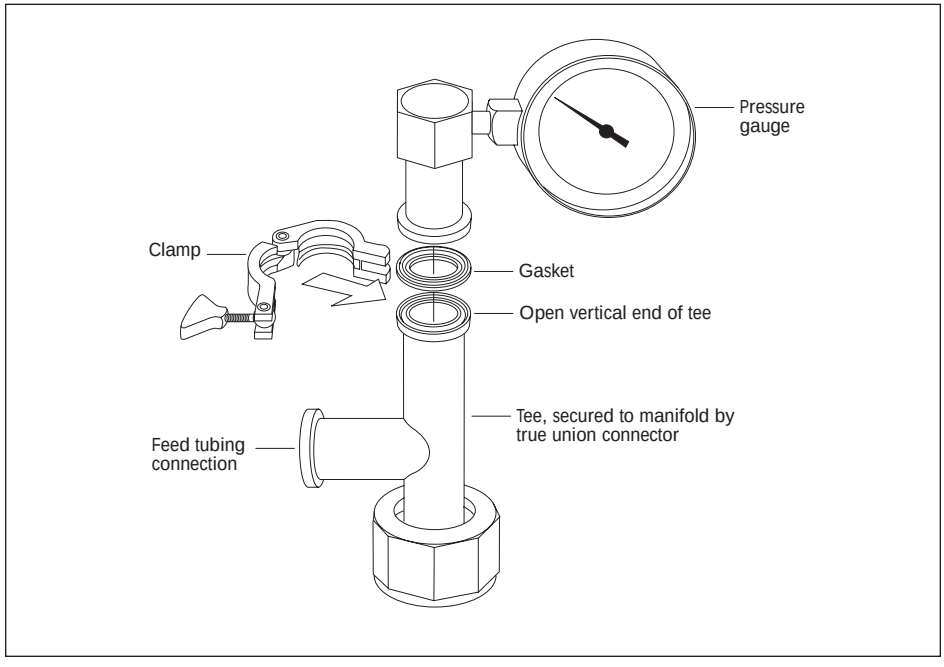

Figure 8. Sequence and Orientation of Gauge, Gasket and Clamp on Tee

a. Take a pressure gauge, a gasket and a clamp.
b. Place the gasket into the groove on the open vertical end of the tee.
c. Fit the pressure gauge onto the open vertical end of the tee, with the gasket.
d. While holding the pressure gauge still, use the clamp to secure the gauge in place and to make a water-tight closure.

Attaching the Pressure Gauges to the Manifold / Module Assembly, continued

4. Place a gasket into the groove on one of the permeate fittings, on the manifold (see Figure 4). Be sure that the gasket is seated snugly. (This port will be used to monitor permeate pressure.)
5. Install a union nut and ferrule on the fitting end of the second pressure gauge.
   a. Slide the union nut and the ferrule over the fitting end of the pressure gauge, as shown in Figure 9.

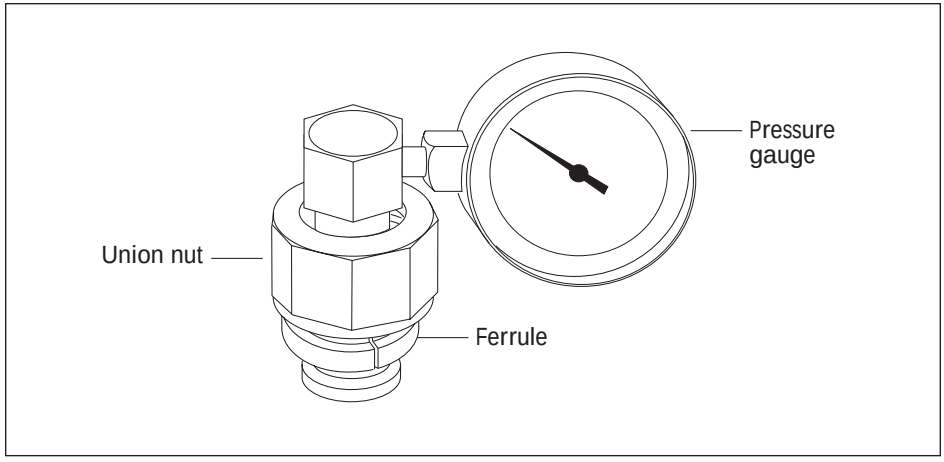

Figure 9. Union Nut and Ferrule on Pressure Gauge

   b. Compress the ferrule, so that it slides into the union nut.
   c. Push the union nut downward over the fitting end of the pressure gauge until it forms a snug cap.
6. Install the gauge on the chosen permeate fitting of the manifold.
   a. Place the gauge, with the union nut and ferrule, over the permeate fitting, with its gasket.
   b. Screw the combined union nut and ferrule onto the split nut until finger-tight.

Attaching the Tubing to the Manifold/Module Assembly

As in the previous installation of the tee and a pressure gauge, true-union connectors will be used to connect tubing to the remaining feed/retentate and permeate ports on the manifold (as shown in Figure 4).

The third piece of tubing will be attached to the open fitting of the tee on the feed/retentate port, using a stainless steel clamp.

The feed, retentate, and permeate tubing can be identified by size. The feed tubing has a T-connector with an attached diafiltration line. Table 1 shows the sizes of the tubing for each of the three module sizes, the nominal areas of which are repeated below:

Module	**Nominal Membrane Area**	
one-stack	150 cm^2	(1/6 ft^2)
two-stack	0.03 m^2	(1/3 ft^2)
six-stack	0.1 m^2	(1 ft^2)

Table 1. Tubing Sizes

Tubing Element	**Number of Stacks in Module**	**O.D.** mm (in.)	**I.D.** mm (in.)	**Length** cm (in.)
Feed (plain)	1 and 2	7.9 (5/16)	4.7 (3/16)	73.7 (29)
Feed (diafilter, attached with T-connector)	1 and 2	7.9 (5/16)	4.7 (3/16)	81.3 (32)
Retentate	1 and 2	7.9 (5/16)	4.7 (3/16)	61.0 (24)
Permeate	1 and 2	6.3 (1/4)	3.2 (1/8)	71.1 (28)
Feed (plain)	6	9.5 (3/8)	6.3 (1/4)	86.4 (34)
Feed (diafilter, attached with T-connector)	6	9.5 (3/8)	6.3 (1/4)	94.0 (37)
Retentate	6	9.5 (3/8)	6.3 (1/4)	61.0 (24)
Permeate	6	6.3 (1/4)	3.2 (1/8)	71.1 (28)

Attaching the Tubing to the Manifold / Module Assembly, continued

The ferrule and union nut are installed on the sanitary fitting end of a piece of tubing to form a fitting, which screws onto the split nut, making a sanitary connection between the tubing and the manifold. (For reference, the three parts of the true-union connector are shown in Figure 5.)

1. Install a union nut and ferrule on the fitting end of both the permeate and the retentate tubing.
   a. Slide the union nut and the ferrule over the fitting end of the tubing in the sequence and orientation shown in Figure 10.

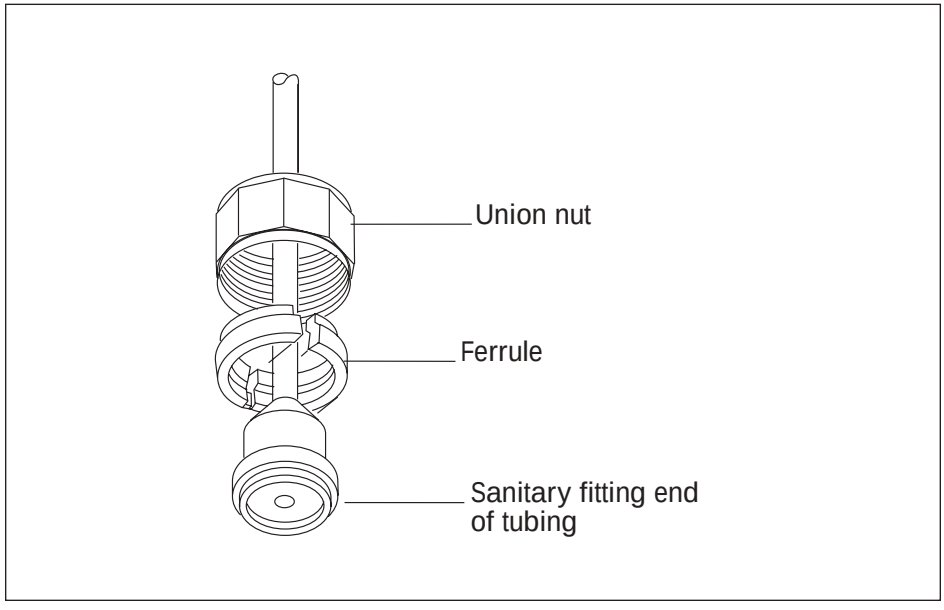

Figure 10. Sequence of Ferrule and Union Nut on Tubing

   b. Compress the ferrule so that it slides into the union nut.

Attaching the Tubing to the Manifold/Module Assembly, continued

c. Push the union nut downward over the fitting end of the tubing (as shown in Figure 11) until it forms a snug cap. You will hear it "click" into place.

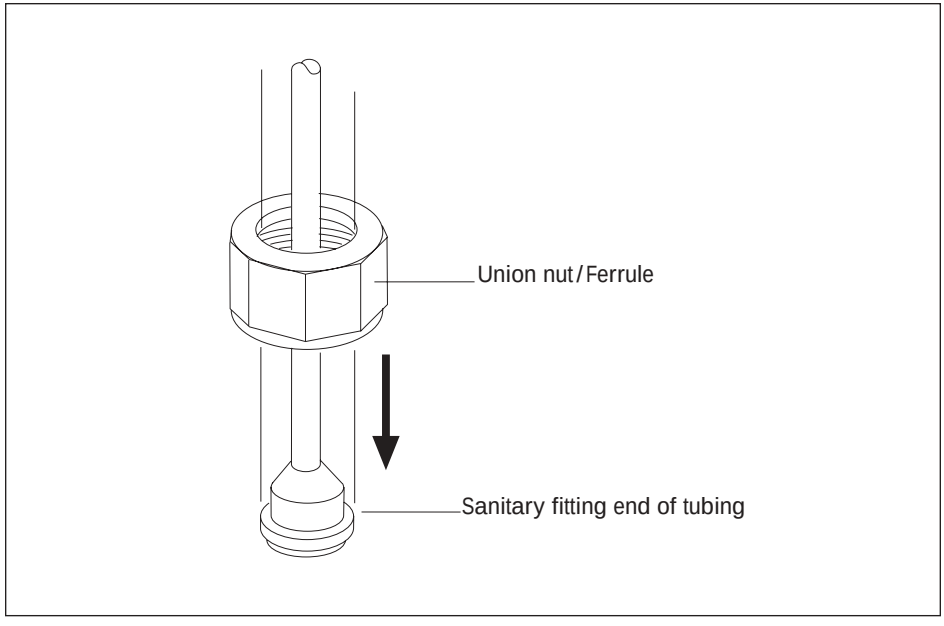

Figure 11. Union Nut and Ferrule on Tubing

2. Install the tubing on the module in the appropriate retentate and permeate positions (see Figure 4), by screwing the union nut onto the split nut. Tighten the connection to finger-tight.
3. Using a stainless steel clamp, attach the feed tubing to the open end of the tee, which is perpendicular to the main body of the tee. (The feed tubing connection is indicated in Figure 8.) The sanitary fitting end of the tubing incorporates a gasket: the only piece needed to attach the tubing to the tee is the clamp.

Attaching the Tubing to the Manifold / Module Assembly, continued

Figure 12 represents the completed assembly of the manifold, module, tubing and gauges.

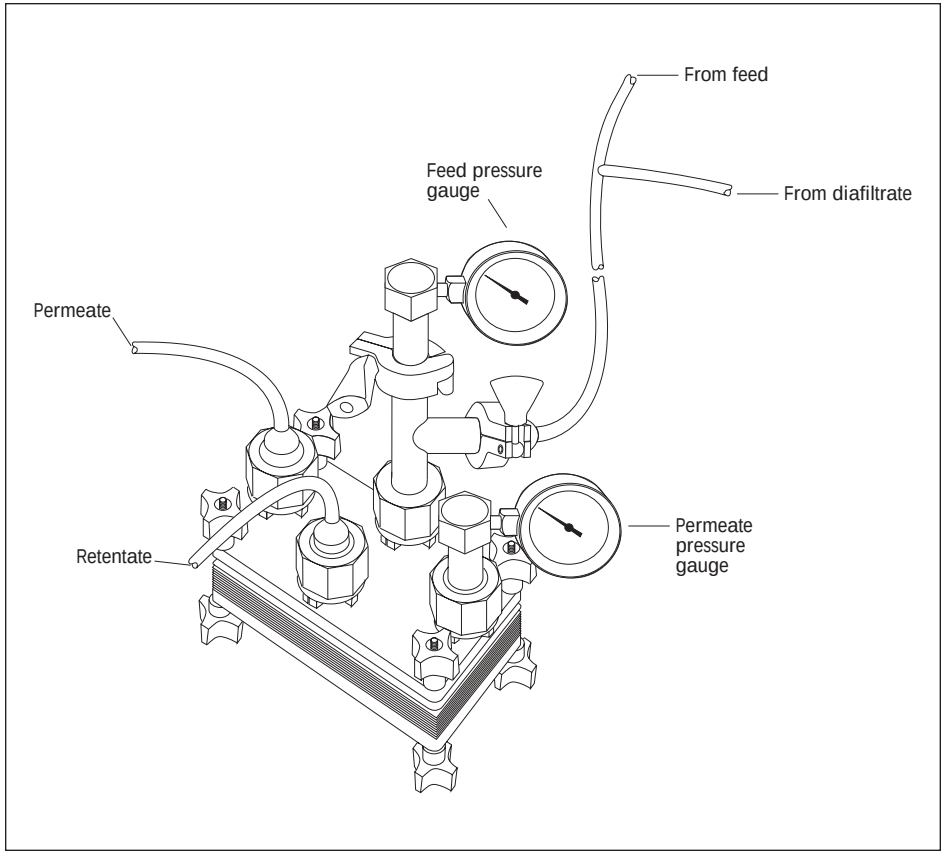

Figure 12. Completed Assembly of Viresolve Virus Removal System

Optional Alternative: Installing the Minicap on the Second Permeate Fitting (instead of a pressure gauge)

During routine operation, it is highly recommended that a pressure gauge be installed on the second permeate fitting, to augment the process monitoring provided by the feed pressure gauge. (The installation of the two pressure gauges was described earlier.) Refer to Figure 4 for the location of the permeate fittings on the manifold/module assembly.

When process monitoring is not critical, the second permeate fitting may be capped with a minicap (provided with the Manifold Kit), as described below. The installation sequence is shown in Figure 13.

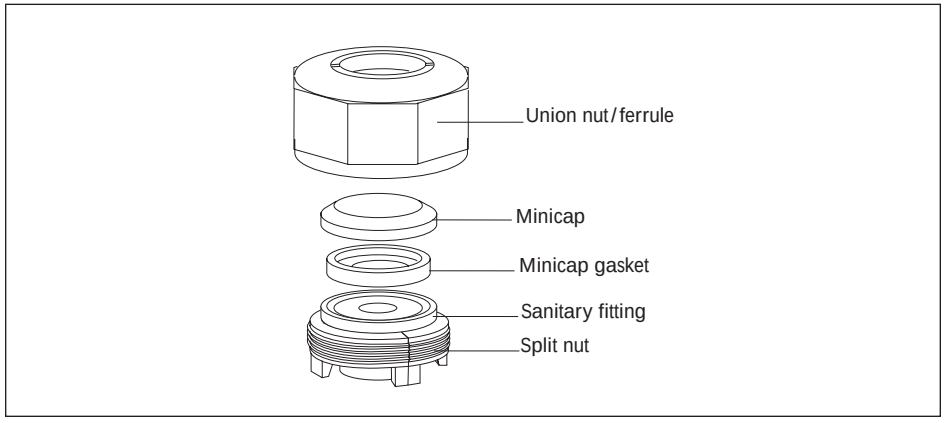

Figure 13. Installation of Minicap

1. Place the minicap gasket into the groove on the second permeate fitting, on the manifold. Be sure that it is seated snugly.
2. Place the minicap, grooved side down, onto the fitting.
3. Install a union nut and ferrule on the minicap.
   a. Compress the ferrule, so that it slides into the union nut.
   b. Screw the combined union nut and ferrule over the minicap, onto the split nut, until finger-tight.

3

Sterilization

Overview

This chapter provides information on:

- Initial sterilization of the Viresolve manifold/module assembly
- Sterilization of a previously water-wet Viresolve manifold/module assembly

Introduction

Viresolve modules are not sterile, although they are packaged in a controlled environment and have been released with an endotoxin level of <0.5 EU/mL. If sterilization is not required, proceed to Chapter 4, Operation and Disassembly.

The Viresolve membrane may be autoclaved **only** when the membrane has water in the pores prior to autoclaving. If the Viresolve membrane is dry when autoclaved, significant flux reduction will result from autoclaving. The entire Viresolve assembly (shown in Figure 12, in the previous chapter) is autoclavable, including the gauges. The Viresolve module itself may be autoclaved only once.

Your autoclave cycle must be validated, to ensure that the Viresolve module, manifold and tubing will be exposed to the indicated temperature for sufficient time, to achieve sterility.

Materials and Equipment

- Assembled Viresolve manifold/module assembly, with gauges and tubing (see Chapter 2 for assembly instructions)
- Clean dry air at 10 psig minimum (microporous-filtered, oil-free)
- Water for injection (WFI) or ≤10,000 MW Milli-Q™ UF water at 16 – 27 °C
- Pressure dispensing vessel (2 liter minimum)
- Pressure test gauge (0 – 20 psig)
- Self-relieving air pressure regulator (0 – 20 psig)
- Autoclave (must be capable of performing a liquid cycle with slow exhaust)

Procedure

1. Place the assembled Viresolve manifold/module assembly, with gauges and tubing, onto a bench.
2. Fill the pressure dispensing vessel with WFI or Milli-Q UF water (at 16 – 27 °C).
3. Attach the pressure dispensing vessel to the module feed port with the installed feed tubing.
4. Set the pressure vessel to 5 psig.

Procedure, continued

5. Allow the WFI or Milli-Q UF water to flow from the retentate port, until all entrapped air has escaped.
6. Clamp off the retentate port, using the clamps provided on the tubing.
7. Set the pressure vessel to 10 psig.
8. Allow the WFI or Milli-Q UF water to flow through the permeate port for 5 minutes.
9. After 5 minutes, stop the water flow through the permeate port, by relieving the pressure in the pressure dispensing vessel. Attempt to keep the tubing filled with water. The membrane **must** have water in all its pores to prevent flux reduction.
10. Disconnect the feed tubing from the pressure vessel.
11. Attach approved steam-porous coverings on tubing outlets.
12. Place the manifold/module assembly with the attached tubing into the autoclave. The manifold/module assembly should be in a horizontal (water-holding) position, with the sanitary fittings facing up.

    NOTE: The module **must** remain in the manifold during autoclaving.
13. Place the ends of the tubing into a beaker containing water, in the autoclave.
14. Set the autoclave for a “liquid” cycle (with no vacuum). Do **not** use a “wrapped good” or “hard good” cycle.
15. Set the autoclave for “slow exhaust.”
16. Autoclave at 121 °C for 60 minutes.
17. After the autoclave cycle is complete, let the manifold/module assembly cool for at least 30 minutes before use.

Autoclaving Procedure for Previously Water-wet Modules

If you are sure that the Viresolve membrane has not dried out since prior wetting with water, autoclave the assembly, as described in Steps 11 through 17 in the preceding procedure.

If there is any doubt whether the membrane has dried out, begin with Step 1 in the preceding procedure.

4

Operation and Disassembly

Overview

This chapter provides information on:

- Connecting the tubing to the pumps and reservoirs
- Operating the Viresolve Virus Removal System
- Disassembly of the system, following operation
- Sterilizing the reusable components

Connecting the Tubing to the Pumps and Reservoirs

Loading the Tubing into the Pump Heads

The configuration of the tubing and the pumps is shown in Figure 14.

1. Thread the free end of the feed line through the lobes of the recirculation pump, according to the directions for your specific type of pump. If using a feed line with a diafiltration loop, the T-connector of the feed line should be upstream of the pump. Do **not** start the pump.
2. Thread the free end of the permeate line through the lobes of the permeate pump, according to the direction for your specific type of pump. Do **not** start the pump.

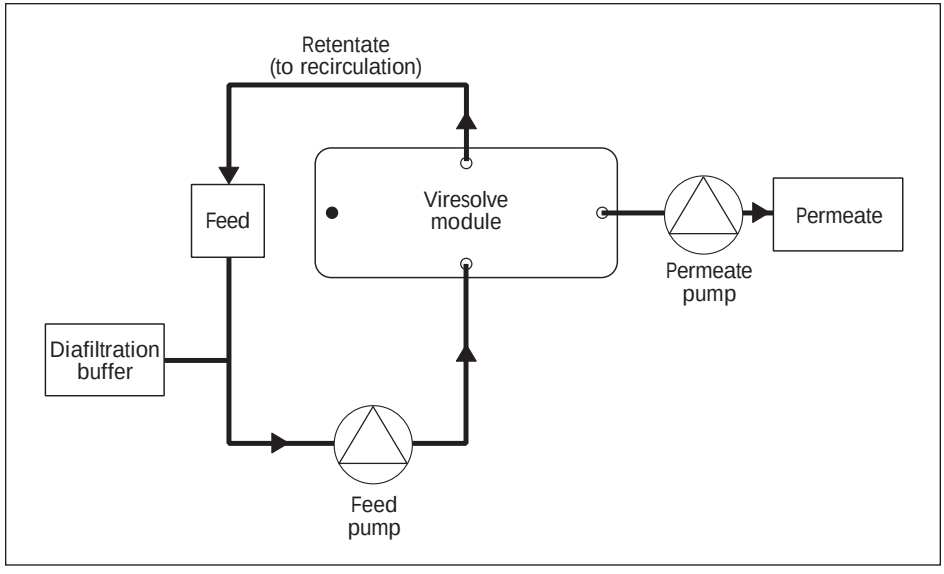

Figure 14. Configuration of Tubing and Pumps

Connecting the Tubing to the Feed and Collection Reservoirs

1. Attach the free end of the feed inlet to the feed reservoir outlet.
2. Attach the free end of the retentate line to the feed reservoir inlet.
3. Attach the free end of the permeate line to the collection reservoir.

Operating the Viresolve Virus Removal System

This procedure is very general and must be adapted for your application. For more information on optimizing operating conditions, see Chapter 5, Optimization.

1. Confirm that the permeate line is in the collection vessel.
2. Turn on the feed pump and allow the feed to recirculate for 15 – 30 minutes. (A small amount of product may flow into the collection vessel. If a significant amount begins flowing prior to 15 – 30 minutes, turn on the permeate pump and begin processing at a flow rate appropriate for your batch collection.)
3. Turn on the permeate pump. Process the product until the minimum hold-up volume is achieved. The recommended minimum hold-up volume will vary, dependent on your tubing configuration. For many applications, the minimum hold-up volumes are:

One-stack modules	18 – 23 mL
Two-stack modules	30 – 35 mL
Six-stack modules	150 mL

4. If desired, diafilter the recirculated retentate with an appropriate buffer.

Disassembly following Operation

1. When all the product has been processed, flush the system with clean water or a sanitization agent.
2. Clamp off all tubing to feed, buffer and product. Do not detach the tubing from the manifold.
3. Autoclave the manifold/module assembly, with tubing and gauges still attached, at 121 °C for 30 minutes.
4. Unscrew or unclamp the tubing and gauges from the manifold/module assembly. Remove the union nuts, ferrules, gaskets and tee and set aside.
5. Remove the minicap (if used), gasket and true union connector and set aside for cleaning.
6. Remove the wingnuts and set aside for cleaning.
7. Remove the upper manifold plate and set aside for cleaning.

   NOTE: In the next step, it is very important to keep the module upright. Do **not** tilt the module or turn it upside down. This may cause drainage of upstream contaminants.

8. Keeping it upright, carefully remove the module from the manifold.
9. Autoclave and dispose of the module and tubing, according to the customary requirements of your application.
10. Remove the thumbscrews from the lower manifold plate.

Sterilizing the Reusable Components

Autoclave the manifold plates, true union connectors, minicap, gauges, clamps, gaskets, tee, wingnuts and thumbscrews at 121 °C for 30 minutes.

5

Optimization

Overview

This chapter provides information on:

- Optimizing operation of the Viresolve Virus Removal Module for your specific application
- Estimating the surface area required for scale-up

Introduction

This protocol enables you to optimize the operation of the Viresolve system for your specific application, based on the results of a trial using the 1/6 ft^2 (150 cm^2) one-stack module. The purpose of this trial is:

- To evaluate protein mass and activity-sieving coefficients as a function of operating conditions
- To determine optimal operating conditions
- To estimate the surface area required for scale-up

Two types of experiments, flux excursion and volume reduction, are performed to obtain this information.

The flux excursion experiment is performed with concentrated product, to evaluate protein-sieving coefficient as a function of permeate flow rate (at a constant cross flow rate), as well as any concentration effects. (It is assumed that filtration efficiency can be monitored by measuring total protein at $A_{280\ nm}$ on a spectrophotometer.)

Once the optimal conditions are determined by the flux excursion experiment, a volume reduction is performed. The volume reduction experiment is a process run; a product batch is filtered until the hold-up volume is reached. A diafiltration step to recover at least the original volume is performed, with additional diafiltration if necessary for protein mass recovery.

Based on the optimal conversion calculated from these accumulated data, scale-up estimations can be determined. The 1/6 ft^2 (150 cm^2) one-stack module is the preferred size for this experiment, due to its low feed volume requirement. For assistance with optimization on larger modules, please contact your Millipore Applications Specialist.

Terminology

Permeate (P)	purified product that passes through the membrane
Retentate (R)	material retained by the membrane
Conversion (C)	permeate flow rate/cross flow rate
Flux (J)	flow rate/area
Mass Flux (G)	optimal productivity (% passage) from membrane area (m^2) per unit time (hours) and protein concentration (g/L)
Sieving Coefficient (s)	protein content of permeate/ protein content of retentate
Transmembrane Pressure (TMP)	applied pressure across the membrane

Data Collection and Analysis

It is recommended that the transmembrane pressure (TMP), absorbence at A_{280nm} and flow rate be recorded at each step in this protocol. The table on the following page is provided to assist data collection and analysis.

When two pressure gauges are used (as recommended), transmembrane pressure is calculated using the following equation:

$$TMP = P_{Feed} - P_{Permeate}$$

Process flux, J (flow rate/area), is typically expressed in the following terms: $L/m^2/hour$ (frequently referred to as LMH).

If flow rate has been recorded in terms of **mL/min**, and area has been specified in terms of **cm^2**, then process flux (J) in terms of **$L/m^2/hour$** or **LMH** may be calculated using the following conversion equation:

$$J = \text{Flow Rate (mL/min)} \times \frac{1 \text{ liter}}{1000 \text{ mL}} \times \frac{10{,}000 \text{ cm}^2}{1 \text{m}^2} \times \frac{1}{\text{Device Membrane Area (cm}^2\text{)}} \times \frac{60 \text{ min}}{1 \text{ hour}}$$

Data Collection and Analysis Table

Sample No.	Absorbence (A_{280nm})	TMP (psi)	Flow Rate (mL/min)	Process Flux (LMH)

Flux Excursion Protocol

1. Fill the feed bag (reservoir) with concentrated product (a minimum of 150 mL), from which a 1 mL sample has been removed (to determine the protein concentration at $A_{280\ nm}$). Assemble the system as shown in Figure 15.

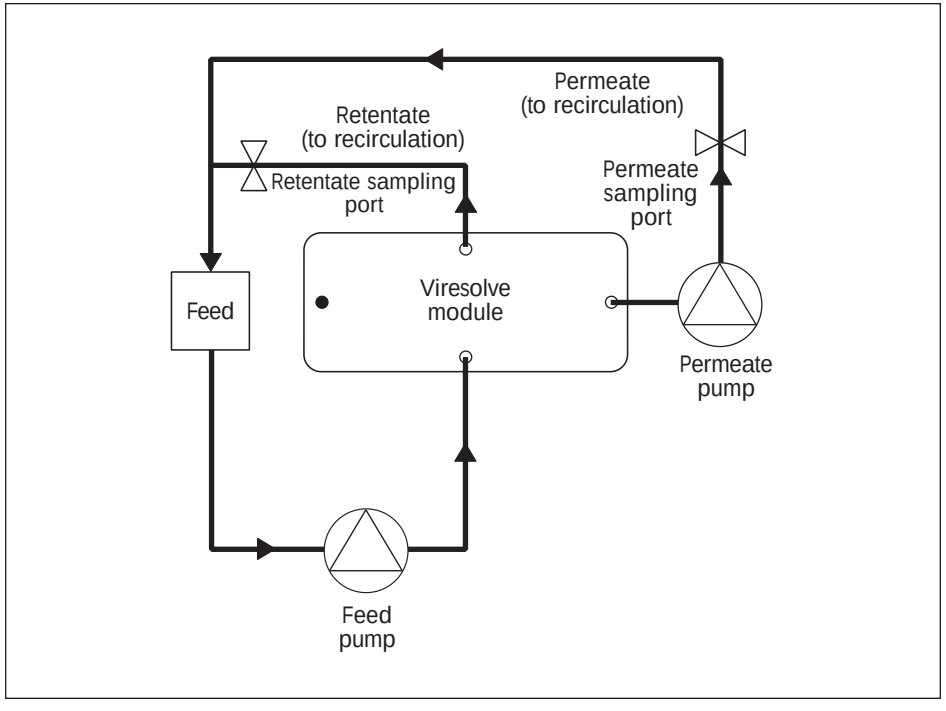

Figure 15. System Configuration for Flux Excursion

Flux Excursion Protocol, continued

2. Turn on the recirculation pump, ramping slowly to prime the module and free the lines of air.
3. Set the recirculation pump flow rate to 250 mL/min. Allow the product to recirculate over the membrane for 30 minutes.
4. Draw a 1 mL sample from the retentate port (R_0) to test for protein adsorption to the membrane, using $A_{280\ nm}$.
5. Run the permeate line and the retentate line back into the feed, for total recirculation.
6. Turn on the permeate pump, to achieve a flow rate of 0.86 mL/min.

   With an Effective Filtration Area of 171 cm^2 (i.e., using the one-stack module), this will result in a flux of:

   $J = 0.005\ mL/min/cm^2$

   This may be converted into L/m^2/hour or LMH, using the conversion equation on the previous page.

   For the specified Effective Filtration Area of each of the available module sizes, see the Specifications section in Chapter 6, Reference Information.
7. Operate in total recirculation (with the permeate and retentate being returned to the feed) for 30 minutes.
8. After the 30-minute recirculation, collect the first permeate and retentate samples (P_1 and R_1, 1.0 mL each), to test for protein adsorption to the membrane, using $A_{280\ nm}$.
9. Increase the flow rate of the permeate pump by a small increment and allow a 30-minute equilibration time with total recirculation.
10. Collect permeate and retentate samples (P_2 and R_2, 1 mL each) to test.
11. Repeat steps 9 and 10 until all desired permeate flow rates (fluxes) have been tested. (The flux values and number of fluxes tested will be dependent upon the protein tested and the sieving results obtained for that specific protein.)
12. Determine the total protein concentrations of the permeate and retentate port samples, using $A_{280\ nm}$. Using an effective filtration area of 171 cm^2 and the tested permeate flow rates, calculate process flux, LMH (liters/m^2/hour), for each 30 minute recirculation time interval.

13. Using the permeate and retentate concentrations calculated in step 12, determine the percent protein passage that occurred for each flux interval. Then take the bulk protein sample concentration, [Protein], from step 1 (in grams per liter) and calculate the total grams of protein for each flux interval, using the mass flux equation:

    $$G = [\text{Protein}] \times \text{LMH} \times \% \text{ passage}$$

14. Graph mass flux (total grams), **G**, on the y-axis and process flux, **LMH**, on the x-axis.

    An example of a Mass Flux Versus LMH curve is shown in Figure 16. The highest point on the curve, indicated by the star, is where the process is optimized for mass flux. In order to maximize the mass flux for your process, it is important to evaluate protein concentration and passage, as is shown in the mass flux equation.

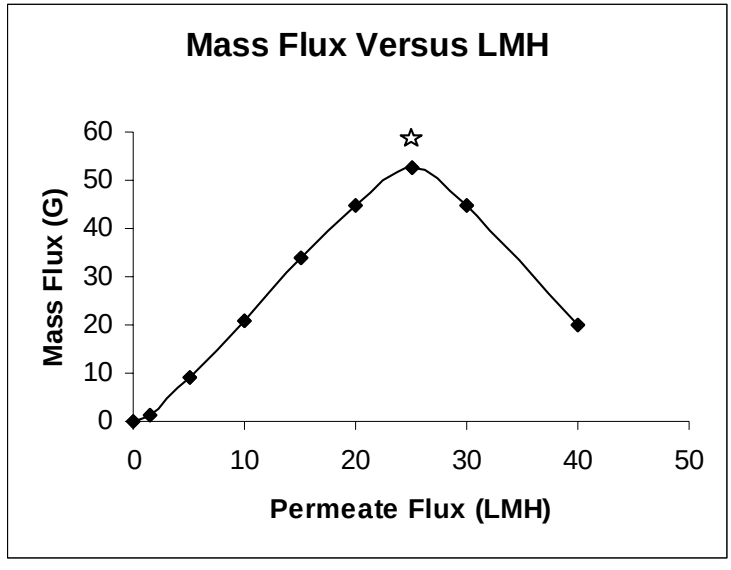

Figure 16. An Example of a Mass Flux versus LMH Curve

Volume Reduction Protocol

Based on the results of the concentrated product flux excursion, the volume reduction experiment is performed at the concentration that will be used in the actual process.

1. Fill the feed bag (reservoir) with the protein (at least 300 mL). Assemble the system as shown in Figure 17.

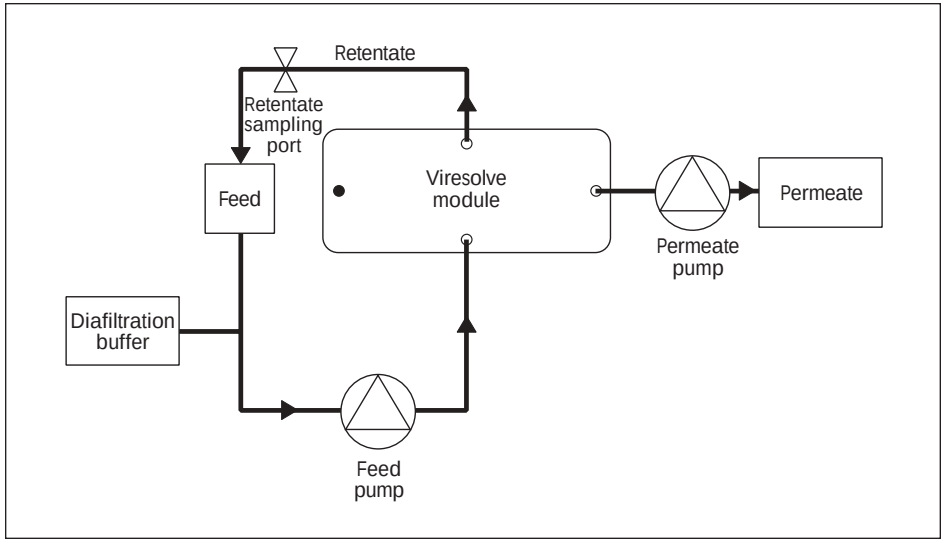

Figure 17. System Configuration for Volume Reduction

Volume Reduction Protocol, continued

2. Turn on the recirculation pump, ramping slowly to prime the membrane module and free the lines of air.
3. Set the recirculation pump flow rate to 250 mL/min. (or the appropriate cross flow rate, as determined by the flux excursion experiment). Recirculate the product for 30 minutes.
4. Process the batch volume at the optimal flux determined by the flux excursion experiment, until the minimum hold-up volume is reached (< 30 mL, dependent upon the tubing length and product reservoir size).
5. Test the protein concentration to determine the actual product recovery **without** diafiltration.

   (Additional retentate and permeate samples may also be drawn during the experiment to check for decreases in sieving coefficient, if desired.)
6. Diafilter with an appropriate buffer at three times the hold-up volume (or more), to recover as much protein mass as possible, without significant product dilution.
7. Check the protein concentration of the diafiltered product to determine whether step 6 will be needed in your process.
8. Calculate the actual product recovery **with** diafiltration.

Determination of Surface Area for Scale-up

Using the mass flux equation, shown in the previous section, it is possible to determine the surface area for scale-up, by keeping the area (m^2) as the unknown and plugging in the other variables for your desired optimized process.

Please consult your Millipore Applications Specialist for assistance with the scale-up of your process.

6

Reference Information

Overview

This chapter provides information on:

- Specifications
- Product Ordering Information
- Technical Assistance
- Warranty

Specifications

Required Accessories

See Chapter 1, Introduction

Operating Parameters

Effective Filtration Area (E.F.A.)

module size		E.F.A
one-stack	1/6 ft^2/150 cm^2	0.184 ft^2/171 cm^2
two-stack	1/3 ft^2/0.03 m^2	0.369 ft^2/343 cm^2
six-stack	1 ft^2/0.1 m^2	1.108 ft^2/1029 cm^2

Minimum Working Volume (by module size)

one-stack	1/6 ft^2/150 cm^2	150 mL
two-stack	1/3 ft^2/0.03 m^2	300 mL
six-stack	1 ft^2/0.1 m^2	1000 mL

Approximate Internal Hold-up Volume (by module size)

one-stack	1/6 ft^2/150 cm^2	12 mL
two-stack	1/3 ft^2/0.03 m^2	24 mL
six-stack	1 ft^2/0.1 m^2	70 mL

pH Range 4 – 8

Transmembrane Pressure Approximately 5 psig/0.35 bar at 4 – 37 °C

Typical Operating Pressures < 5 psig/0.35 bar at 4 – 37 °C

Typical Permeate Flow Rates (by module size)

one-stack	1/6 ft^2/150 cm^2	2 – 10 mL/min for 1 cP
two-stack	1/3 ft^2/0.03 m^2	2 – 20 mL/min for 1 cP
six-stack	1 ft^2/0.1 m^2	2 – 60 mL/min for 1 cP

Recommended Retentate Crossflow (by module size)

one-stack	1/6 ft^2/150 cm^2	200 – 400 mL/min for 1 cP
two-stack	1/3 ft^2/0.03 m^2	300 – 1000 mL/min for 1 cP
six-stack	1 ft^2/0.1 m^2	900 – 2500 mL/min for 1 cP

Dimensions

Module

Length		6.95 in/17.7 cm
Width		3.65 in/9.3 cm
Height (by module size)		
one-stack	1/6 ft^2/150 cm^2	0.35 in/0.9 cm
two-stack	1/3 ft^2/0.03 m^2	0.47 in/1.2 cm
six-stack	1 ft^2/0.1 m^2	0.98 in/2.5 cm

Manifold

Length	6.95 in/17.7 cm
Width	3.65 in/9.3 cm

Materials

Module	PVDF (polyvinylidene fluoride)
Manifold	316L stainless steel, electropolished
Gauges (product-contact surfaces only)	316L stainless steel, electropolished
Tubing and gaskets	medical-grade silicone
True union connectors	PVDF
Minicap	PVDF
Tees and clamps	316L stainless steel, electropolished

Sterilization

Module (must be water-wetted)	Autoclavable one time only at 121 °C, for 60 minutes
Manifold kit, tubing, tees, clamps, gaskets and gauges	Autoclavable at 121 °C, for 60 minutes

For the sterilization procedure, see Chapter 3, Sterilization.

For more information, contact Millipore. See Technical Assistance, in this chapter.

Product Ordering Information

This section lists the catalogue numbers for available Viresolve modules and accessories. See the Technical Assistance section for information about contacting Millipore. You can also buy Millipore products on-line at www.millipore.com/purecommerce.

Description		Qty. /Pk.	Catalogue No.
Viresolve/70 modules			
one-stack	1/6 ft^2/150 cm^2	1	VMVA 01B N1
two-stack	1/3 ft^2/0.03 m^2	1	VMVA 02B N1
six-stack	1 ft^2/0.1 m^2	1	VMVA 06B N1
Viresolve/180 modules			
one-stack	1/6 ft^2/150 cm^2	1	VMVG 01B N1
two-stack	1/3 ft^2/0.03 m^2	1	VMVG 02B N1
six-stack	1 ft^2/0.1 m^2	1	VMVG 06B N1
Accessories			
Viresolve Manifold Kit		1	VMSP 000 02
Easy-Load Pump Head, 1.6 LPM		1	XX80 EL0 05
Variable-Speed Drive, 60 – 600 RPM, 115 V/60 Hz		1	XX82 001 15
Pressure Dispensing Vessel, 1 gallon/3.87 liters		1	XX67 00P 01
Analog Pressure Gauge		1	B26524
CorrTest Kit, 3 × 0.4 L wetting, 3 × 1 L intrusion		1	VMVC T06 S3
Spare Parts			
Viresolve Spare Parts Kit: 4 true union connectors, 1 minicap		1	VMSP A00 01
Viresolve Diafiltration Tube Sets (by module size)			
one-stack	1/6 ft^2/150 cm^2	1	18274
two-stack	1/3 ft^2/0.03 m^2	1	18274
six-stack	1 ft^2/0.1 m^2	1	18276
Wingnut		1	17921
Thumbscrew		1	17922

Technical Assistance

For more information, contact the Millipore office nearest you. In the U.S., call **1-800-MILLIPORE** (1-800-645-5476). Outside the U.S., see your Millipore catalogue for the phone number of the office nearest you or go to our web site at www.millipore.com/offices for up-to-date worldwide contact information. You can also visit the tech service page on our web site at http://www.millipore.com/techservice.

Standard Warranty

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