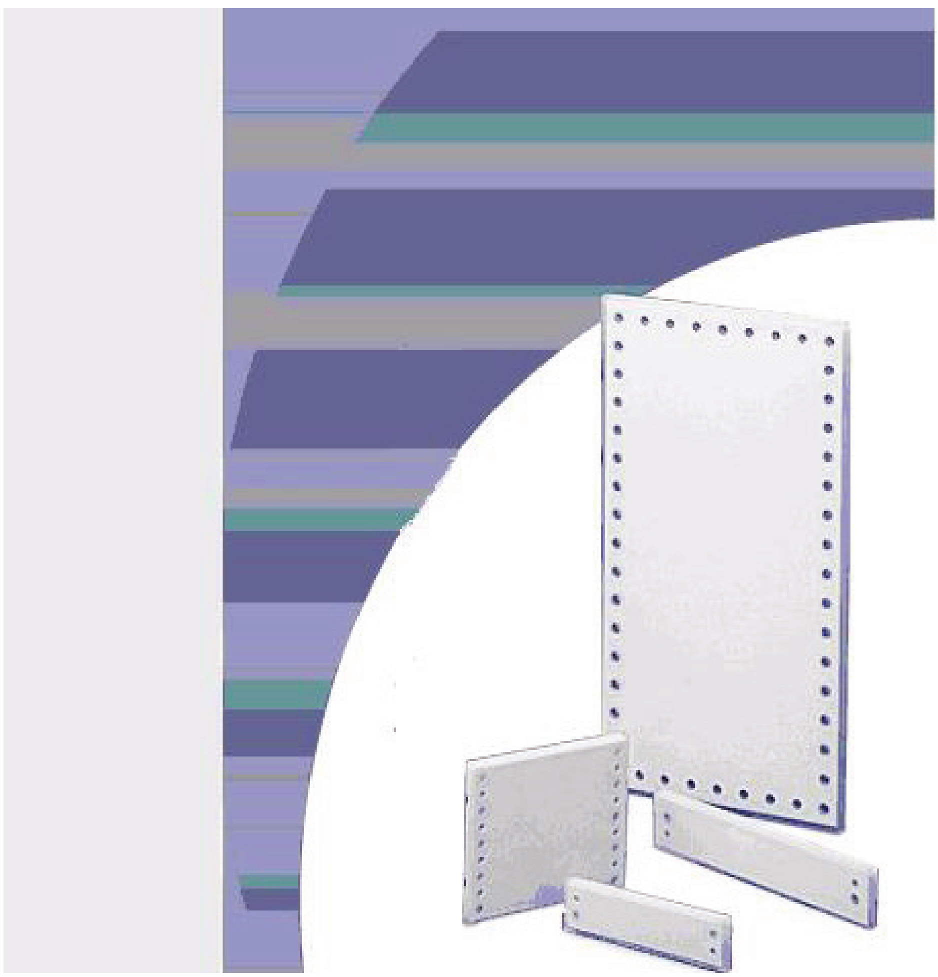
Life Sciences

User Guide

R00640 Rev B 1.00

Membrane Cassette Care and Use Procedures

Centramate™, Centrasette™, Maximate™, Maxisette™ Cassettes



Filtration. Separation. Solution.SM

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Safety Notice

Important Notice
Refer to safety Instructions before use.
Saftey instructions in this language are available from Pall.

Viktigt att notera
Läs säkerhetsinstruktionerna före användandet.
Säkerhetsinstruktioner på svenska finns att få frän Pall.

重要通知
ご使用前に安全にお使いいただくための説明書をお読みください。
日本語でかかれた説明書はポールより入手可能です。

Viktig melding
Les Sikkerhetsinstruksjonen før bruk.
Sikkerhetsintruksjon på norsk vil være tilgjengelig fra Pall.

Belangrijke informatie:
Voor gebruik veiligheids instructies goed doornemen.
Veiligheids instructies in het Nederlands zijn bij Pall verkrijgbaar.

Avvertenza importante
Prima dell'uso leggere le istruzioni per la sicurezza.
Le istruzioni per la sicurezza in Italiano possono essere richieste a Pall.

Aviso importante
Antes de utilzar, consultar instruções de segurança.
Instruções de segurança em Português, encontram-se disponiveis na Pall.

Aviso importante
Antes de usar, consultar Instrucciones de Seguridad.
Instrucciones de Seguridad en este idioma están disponibles por Pall.

Important
Se référer aux instructions concernant la sécurité d'utilisation avant usage.
Les instructions concernant la sécurité d'utilisation sont disponibles en français chez Pall.

Vigtigt
Læs sikkerhedsinstruktioner før ibrugtagning.
Sikkerhedsinstruktioner på dansk kan fås fra Pall.

Tärkeä tiedote
Lue turvallisuusohjeet ennen käyttöä.
Pall toimittaa tarvittaessa suomenkieliset turvallisuusohjeet.

Wichtige Anmerkung
Vor Gebrauch bitte die Sicherheitsrichtlinien lesen. Die Sicherheitsrichtlinien in dieser Sprache erhalten Sie von Pall.

Σημαντική Επισήμανση:
Διαβάστε τις Οδηγίες Ασφάλειας πριν από τη Χρήση.
Οι Οδηγίες Ασφάλειας στα Ελληνικά είναι διαθέσιμες από την PALL.

Learn About Safety

Please read and follow the safety instructions in this User Guide.

Important — Read First

Storage solutions recommended for sanitizing, cleaning, flushing, and storage may be hazardous or corrosive. Follow proper safety procedures when preparing, mixing, and handling these reagents. Refer to Material Safety Data Sheets (MSDS) — available from your supplier — to learn about the specific characteristics, necessary precautions, and suitable remedies for each reagent used.

1. Always wear protective clothing including safety glasses and gloves when working with membrane cassettes, equipment, samples, and reagents.
2. Provide sufficient space for assembling all system components and operating the system.
3. Disconnecting a system component or dismantling an installed cassette holder without first isolating and depressurizing it can result in personal injury and equipment damage. Depressurize a TFF System and cassette holder before dismantling any component.
4. Some system components may be very heavy. Take proper precautions when moving or lifting equipment to prevent personal injury. In some cases, hoists or other lifting equipment may be required.
5. You should always use pressure gauges or pressure sensing devices in your system so you can monitor the system pressure and differential pressure across the membrane cassette.
6. Take suitable precautions before sterilizing the holder with stream or using the holder with hot fluids.
7. Do not use your cassette holder as a coded pressure vessel.
8. Wipe up spills promptly to prevent injury from contact or slipping.
9. Complete the following safety procedures:
   a. Read about the operating limits of the membrane cassette and the proper methods for use detailed in this ***Membrane Care and Use Guide***.
   b. Ensure that your process and cleaning conditions do not exceed the operating limits of the cassette holder, membrane cassette, and sealing materials.
   c. Check that your process equipment and the cassette holder meet local safety codes.
   d. Inspect the holder and seals regularly to detect damaged components.

Safety Conventions in this Manual

Safety information is identified in this instruction manual by the following convention:

Information

Identifies important information about the current topic.

Caution

Caution: Identifies a situation that may cause product damage and may pose a safety risk that can cause personal injury.

Warning

Warning: Identifies a dangerous or potentially dangerous situation that may cause irreversible damage to equipment and poses a safety risk that can cause serious personal injury.

1. Introduction

1.1 Applications in TFF

Tangential Flow Filtration (TFF) is an efficient method for concentrating, desalting, or exchanging buffer solutions of biomolecules ranging in volume from several milliliters to thousands of liters. It can be used to fractionate large from small biomolecules, depyrogenate buffer solutions, harvest cell suspensions, and clarify fermentation broths and cell lysate.

TFF is utilized to perform various steps on a wide range of applications in the biopharmaceutical industry, such as:

- Concentration and desalting of solutions of proteins, peptides and oligonucleotides
- Purification and recovery of antibodies or recombinant proteins from cell culture media
- Clarification of cell lysate or tissue homogenates
- Recovery of products expressed into the media from cell culture
- Preparation of samples (concentrate, desalt, or buffer exchange) before or after column chromatography

1.2 Tangential Flow Filtration Process

The process conditions for using membrane cassettes in a Tangential Flow Filtration system depend on the specific application. Important process parameters that must be considered include:

- Retentate Flow Rate (Cross Flow Rate)
- Transmembrane Pressure
- Temperature
- Product characteristics (such as concentration, viscosity, and additives)

Variations in any of these parameters can affect the quality and reproducibility of the TFF process.

The operating conditions for any TFF process must be established by performing trials, evaluating results, and then modifying conditions as necessary to achieve the required results.

Because tangential flow filtration membrane cassettes and assemblies are used in repeated processing modes, proper care and use is crucial to ensure reliable and reproducible performance from run to run, and to maximize the life of the membrane cassettes.

This manual contains detailed steps for the proper installation, preparation, cleaning and storage of a tangential flow filtration (TFF) membrane cassette and assembly. The procedures include protocols for Pall® screen channel, and suspended screen channel cassettes with ultrafiltration (UF) or microfiltration (MF) membranes. Some processes may require adaptations to suit the specific requirements of a given system configuration or application, but essential protocols should not be omitted.

To perform these procedures, it is recommended that the TFF assembly include 3 pressure gauges/transducers and 2 valves for proper execution of the protocols presented, see Figure 2: Typical TFF Hardware Setup. To include integrity testing capability (highly recommended), additional valves may be required (Figure 16: System Setup for Determining Air Integrity).

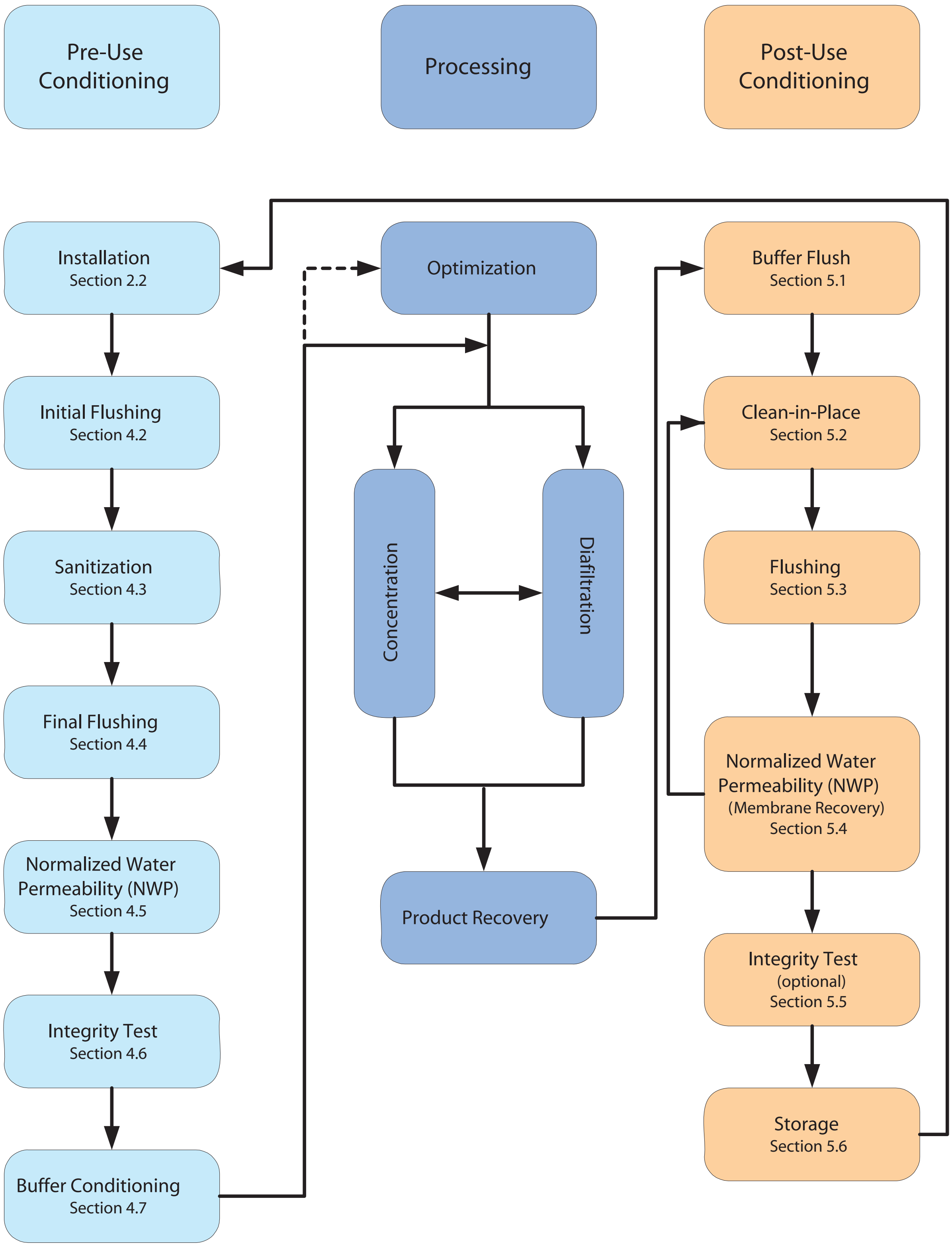
1.3 The TFF Process

The steps required in a Tangential Flow Filtration process are displayed in Figure 1: The TFF Process. Preconditioning and post-use conditioning are covered in Sections 4. and 5. of this manual.

Information on product process optimization and processing can be found in other supporting literature or through Pall Life Sciences Technical Support:

PN33289 *Diafiltration: A Fast Efficient Method for Desalting or Buffer Exchange of Biological Samples*
PN33213 *Introduction to TFF for Laboratory and Process Development Applications*

Figure 1: The TFF Process



1.4 System Hardware Configurations

System hardware configurations vary, but should all have key components (Figure 2: Typical TFF Hardware Setup).

All TFF systems should have:

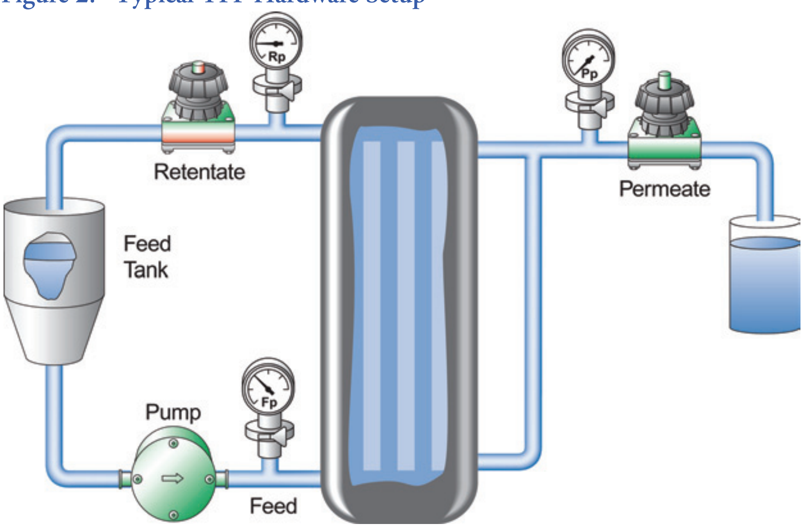
Feed/Retentate Flow Path

- sample/feed reservoir
- feed pump
- feed and retentate pressure gauges or transducers
- adjustable retentate valve
- connecting piping

Permeate Flow Path

- pressure gauge/transducer
- valve

Figure 2: Typical TFF Hardware Setup



1.4.1 Options

In addition to the basic configuration, a valve on the feed side may be added to isolate the system for integrity testing or to separate it from the feed tank. A flow meter is also typically included with larger systems to allow feed or retentate flow rate measurement. A second flow meter may be used on the permeate. Alternatively, the permeate receptacle may be placed on a balance. The weight of permeate collected can be used to calculate permeate volumes.

Temperature sensors may also be located at the feed tank, on the feed line after the pump, and on the permeate line.

- Measuring temperature right after the pump will indicate if the pump is causing excessive heating and possible denaturing of proteins.
- Measuring the temperature in the feed tank will only show gradual heating.
- Measuring the temperature of the permeate gives the actual temperature of the fluid passing through the membrane for determining normalized water permeability (NWP).

1.4.2 System Design

The specific type, size and location of components in a system can significantly affect the performance and cleanability of the system (elimination of bioburden). Critical evaluation of the system design and components should be performed by qualified design engineers, especially when the system will be used in process development and production and will require appropriate validation. Pall process engineers have extensive experience in TFF system design and are available to help you with your engineering questions.

1.4.3 Ports and Flow Rate

The permeate side of most TFF cassette holders have two ports. While these ports in Pall TFF holders are internally connected through the cassettes, closing off one of the ports can create an internal pressure drop in the cassette at high permeate flow rates, > 100 LMH (L/m^2/hr). These high flow rates are frequently encountered when performing water permeability measurements on the cassettes. Since the pressure drop is before the permeate pressure gauge/transducer, it is not measured by the system. This results in a transmembrane pressure (TMP) measurement/calculation (Equation 1: Transmembrane Pressure Calculation) that is in error on the high side resulting in a low calculated value for water permeability (Equation 2: Water Permeability Calculation). In applications where the process is controlled by TMP, the result may be a low process flux rate.

Equation 1: Transmembrane Pressure Calculation

$$TMP = \left(\frac{P_{feed} + P_{retentate}}{2} \right) - P_{permeate}$$

Equation 2: Water Permeability Calculation

$$WaterPermeability = \frac{PermeateFluxRate(LMH)}{TMP}$$

2. Cassette Installation

2.1 First Time Use of Cassette Holder / TFF System

2.1.1 Flush and Clean the System

Before using your TFF system for the first time, flush the system (without cassettes installed) to remove any oil and particulates from the manufacturing process that could damage the cassette or contaminate your product.

Use a detergent-based cleaning solution to clean the system. The solution you choose should be chemically compatible with the components in your system. Typically detergent cleaning solution concentrations between 0.5 and 1.0 percent at temperatures from 25 to 45 °C are used.

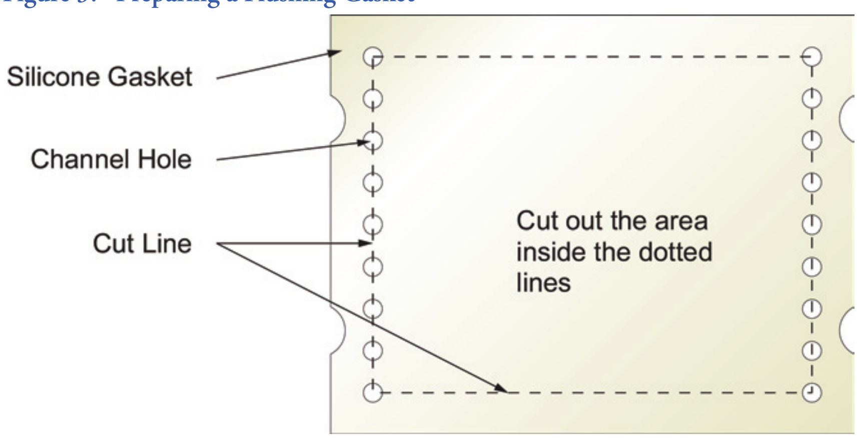
A flushing gasket or SIP/CIP Plate should be installed in the holder in place of a cassette.

You can make a flushing gasket from the silicone gaskets supplied with membrane cassettes. To prepare a flushing gasket, cut the center out of two spare silicone gaskets (also available as spare parts). Use the two gaskets in place of one flushing gasket.

2.1.2 Making a Flushing Gasket

In the generic drawing (Figure 3: Preparing a Flushing Gasket), the dotted line illustrates where to cut the gasket. The cut line should bisect the channel holes while providing about 2 cm (0.75 in.) of space around the outside of the gasket.

Figure 3: Preparing a Flushing Gasket



Purchasing a Flushing Gasket

Flushing gaskets (also called SIP/CIP plates) are also available for purchase. Not supplied with holders.

2.1.3 System Flushing Protocol

1. Fill the feed tank with water.
2. Direct the retentate and permeate lines to drain.
3. Open retentate and permeate valves
4. Pump the water out through both the retentate and permeate lines to drain.
5. Prepare a sufficient amount of detergent-based solution to fill the system flow path and add to feed tank. (You should be able to run the system without drawing air into the pump.)
6. Position both the retentate and permeate lines to return to the feed tank
7. Adjust the pump to provide an aggressive recirculation flow rate. Do not exceed a feed pressure of 0.34 bar (5 psi) Higher pressures can distort the gasket and cause leaks.
8. Allow the detergent solution to circulate through both the retentate and permeate lines for 30 to 60 minutes.
9. Drain the system.
10. Wash and then refill feed tank with water

11. Flush the system thoroughly to drain using deionized water to remove the detergent solution. If caustic detergent was used, measure the pH of the waste streams and continue flushing until the pH matches that of the influent.
12. Drain the system and remove the flushing gasket.
13. Install the cassettes

2.2 Installing Membrane Cassettes into Autotorque (AT) and Manual Torque (MT) Holders

For Maxisette™ Holders see Section 2.3: Installing Cassettes into Maxisette AT and MT Holders on page 7

2.2.1 Preparation for Loading Cassettes

Procedure

1. Remove the retaining nuts, spacers and washers. Separate the sliding end plate from the fixed flow-distribution manifold:
   (i) for horizontal units, lift the end plate off the manifold;
   (ii) for vertical units, slide the end plate away from the manifold about 6 – 9 inches.
2. Inspect and, if necessary, clean the cassette holder sealing surfaces.
   Inspect the cassettes and gaskets prior to installation for any damage or foreign material which could hamper sealing of the cassettes.

2.2.2 Installing Cassettes and Gaskets

Preparation: *Remove the film cover from the gaskets and discard before installation*

Procedure

1. Rinse the silicone gaskets (supplied with the cassettes) with deionized or pharmaceutical grade water. Place a gasket flat against the manifold, aligning the holes in the gasket with the holes in the manifold.
2. Place the cassette into the holder against the gasket.
   Place another gasket flat against the cassette.
   Ensure the holes in the manifold, gaskets, and the cassette line up.
3. If your application requires multiple cassettes, continue the same *gasket-cassette-gasket* pattern, ending with a gasket between the last cassette and the end plate (Figure 4: Typical Installation of Cassettes and Gaskets).

Install multiple cassettes with the printed cassette information all facing the same side and direction. Try to position cassettes so that they can be read without having to reopen the holder.

4. Place or slide the end plate against the last gasket of the cassette stack:
   (i) for horizontal units, place the end plate on top of the cassette stack;
   (ii) for vertical units, slide the end plate against the cassette stack.
5. For hardware assemblies that require tie-rod spacers, (Centramate™, Maximate™, Centrasette™ LV, Centrasette 5), place the spacers and washers on each bolt leaving a minimum of 18 mm (0.75 inches) of thread exposed on the rod. Screw the nut on each bolt and hand tighten firmly.

For auto-torque holders, ensure that the piston is fully retracted into the hydraulic cylinder before hand tightening the nuts. If it is not, push the piston in by hand (with no hydraulic pressure applied to the system).

6. Set the clamping force on the cassettes in the holder to the recommended torque for manual torque (MT) holders or hydraulic pressure for auto-torque (AT) holders according to the instructions found in Section 2.4: How to Torque Pall AT and MT Cassette Holders on page 9.

Two gaskets are supplied with each cassette. Installing the first cassette in a holder requires two gaskets. Installing each additional cassette requires only one gasket. In holders where cassettes are installed on each side of the central manifold, two gaskets will be required for each first cassette. Save extra gaskets to replace worn or damaged gaskets.

Gaskets lose their resiliency over time. Therefore, it is recommended that you replace gaskets every six months, or more frequently if a gasket appears to be damaged or you repeatedly open and close the holder.

2.3 Installing Cassettes into Maxisette AT and MT Holders

Note: Maxisette Manual Holders are no longer available

2.3.1 Preparation for Loading Cassettes

Procedure

1. Unthread the nuts on each 3/4 inch stainless steel bolt until the end of the bolt is reached to allow as much room as possible to install the gaskets and the cassettes. If the cassettes are to be loaded from the side, remove one center-side bolt from the holder. If the cassettes are to be loaded from the top, no bolts need to be removed.
2. Inspect and, if necessary, clean the stainless steel sealing surfaces.
   Inspect the cassettes and gaskets prior to installation for any damage or foreign material that could hamper sealing of the cassettes.
3. Insert or retract the locating pins so they are extended about 10 cm (4 inches) into the holder.

2.3.2 Installing Cassettes and Gaskets

For Maxisette 50 or 100 Holders, the procedure for Installing Cassettes and Gaskets (below) is repeated on both sides of the center manifold. The number of cassettes to be installed should be distributed equally (e.g., for 6 cassettes, use 3 cassettes per side; for 9 cassettes, use 5 cassettes on one side and 4 on the other). On Maxisette 25 holders, cassettes and gaskets are installed only on the side of the manifold plate with the ports.

Procedure

1. Remove the film cover from the gaskets and discard.
2. Rinse the silicone gaskets (supplied with the cassettes) with deionized or pharmaceutical grade water.
   Place a gasket flat against the movable brace plate using the locating pins to position it accurately.
3. Position the cassette on the locating pins and push it in toward the brace plate against the gasket. Place another gasket over the locating pins flat against the cassette.

The cassettes are not position-sensitive from top to bottom or from end to end.
They have identical flow paths regardless of orientation.

4. If your application requires multiple cassettes, continue the same gasket/cassette/gasket pattern, ending with a gasket against the last cassette. Push the locating pins further into the holder as needed to support the next cassette and gasket installed. Make sure the previous gasket has not been wrinkled or damaged.

Install multiple cassettes with the printed cassette information facing all on the same side and direction, and if possible, so that it can be read, without having to reopen the holder.

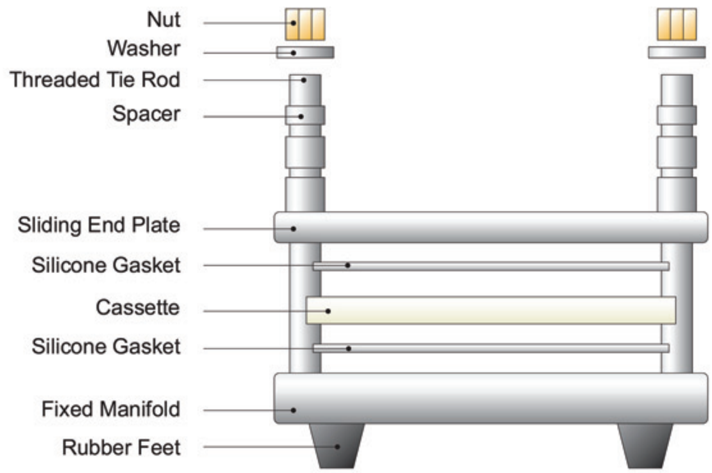
5. After the last cassette and gasket have been installed, close the brace plate by pushing it with both hands at the lowest part of the plate, just adjacent to the rails. This will help eliminate cocking of the plate while closing. Push the locating pins all the way in and leave them in during operation.
6. Reinstall the stainless steel bolt and nut if it had been previously removed.
7. Ensure the pistons are fully retracted into the hydraulic cylinder before hand tightening the nuts. If it is not, push the piston in by hand (no hydraulic pressure on system). Finger-tighten the six nuts down onto the brace plate surface. The holder is now ready to be hydraulically clamped.
8. Set the clamping force on the cassettes in the holder to the recommended hydraulic pressure for auto-torque (AT) holders or adjust the torque for manual-torque (MT) holders according to the instructions in section Section 2.4: How to Torque Pall AT and MT Cassette Holders on page 9

Pall supplies two gaskets with each cassette. Installing the first cassette in a holder requires two gaskets. Installing each additional cassette requires only one gasket. In holders where cassettes are installed on each side of the central manifold, two gaskets will be required for each first cassette. Save extra gaskets to replace worn or damaged gaskets.

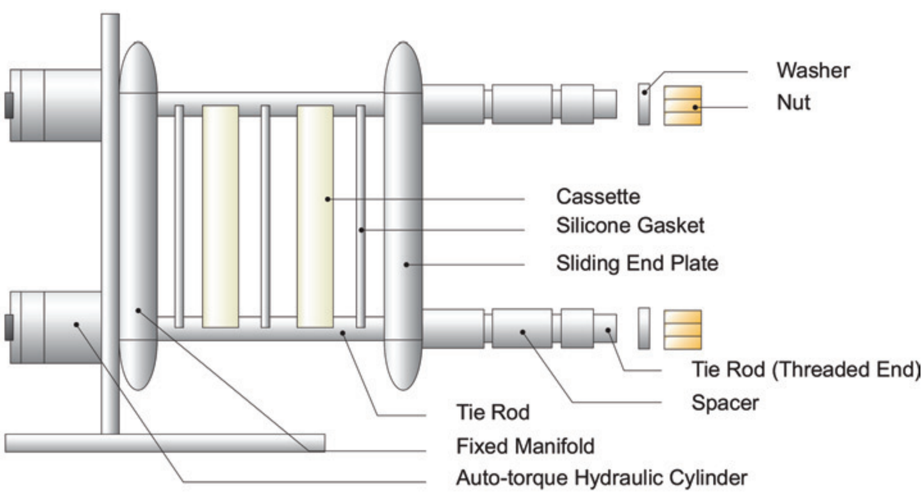
Gaskets lose their resiliency over time. Therefore, Pall recommends that you replace gaskets every six months, or more frequently if a gasket appears to be damaged or you repeatedly open and close the holder.

Figure 4: Typical Installation of Cassettes and Gaskets

Typical installation of cassettes and gaskets in a manually-torqued horizontal unit (top) and in an automatically-torqued, vertical unit (bottom)



NOTE: If your application requires multiple cassettes, continue the same gasket/cassette/gasket pattern, ending with a gasket between the last cassette and the end plate.



NOTE: If you are using more than five cassettes, you should use an intermediate plate and place half of the cassettes on one side of the intermediate plate and half the cassettes on the other side of the intermediate plate. Order intermediate plates from Pall.

Figure 5: Maxisette 50 Diagram Showing Locating Pins

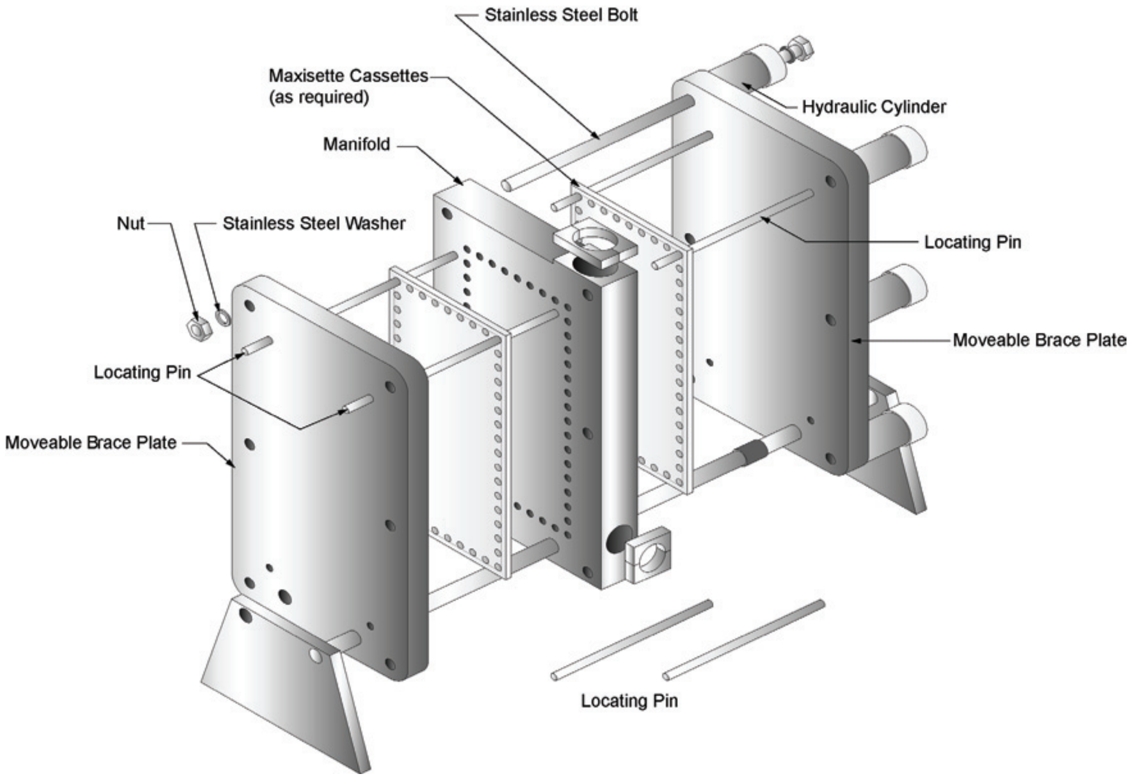
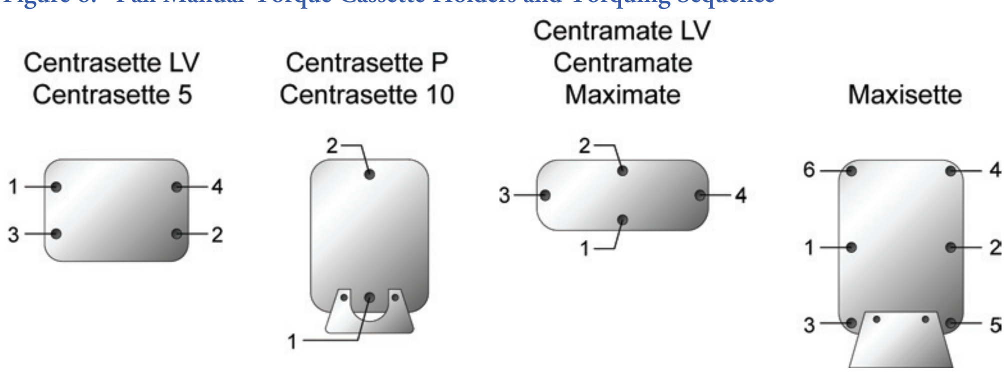


Figure 6: Pall Manual-Torque Cassette Holders and Torquing Sequence



2.4 How to Torque Pall AT and MT Cassette Holders

1. Install the membrane cassette and gaskets in the holder according to Section 2.2 or 2.3, *Installing Membrane Cassettes*.
2. Choose the value at the low end of the hydraulic pressure range for AT assemblies (Table 1: Recommended Hydraulic Pressure Range for Pall AT Cassette Holders on page 11) or the torque range for MT holders (Table 2: Recommended Torque Values for Pall Manual-torque Cassette Holders on page 12) — unless experience has established a different value for use.
3. Compress the holder:

AT Assemblies

(i) Ensure that the nuts are screwed up against the end plate. They need not be tight.
(ii) Close the bypass valve on the hydraulic pump.
(iii) Turn on the air supply to the hydraulic pump.

(iv) Increase the hydraulic pressure on the hydraulic pump until it reaches the pressure value you determined in step 2.

Figure 7: Hydraulic Pump

MT Assemblies

(i) Tighten the nuts firmly by hand.
(ii) Set the calibrated torque wrench (supplied with the cassette holder) to the torque value you determined in step 2. Refer to the instruction sheet for the wrench for additional calibration details.
(iii) Using the torque wrench in the sequence displayed in Figure 6: Pall Manual-Torque Cassette Holders and Torquing Sequence for your holder, tighten each nut no more than 1/4 turn at a time. For example, tighten the first nut 1/4 turn, and then tighten the next nut in the sequence 1/4 turn.
(iv) Stop tightening each nut immediately when the torque wrench "clicks," or the wrench arm pivots slightly away from the socket indicating that the nut has reached the correct torque. Continue torquing each nut in sequence until the torque wrench "clicks" immediately on each nut in sequence. If torqued properly, the nuts should reach the set torque value at about the same time.

The indication that the torque wrench has reached the set value may be very subtle. Therefore, tighten the nuts slowly to prevent exceeding the required torque value.

Note: At this point in the installation, the hold-up volume and minimum working volume should be determined. See Section 3.3: Hold-up Volume and Minimum Working Volume on page 14.

4. After cassettes have been installed and flushed with water, the system should be inspected for leaks. If liquid is found leaking from around the cassettes, increase manual torque in 10% increments or the hydraulic pressure in increments of 3 bar (45 psi). Flush the system with water and check again for leaks. Stop increasing the hydraulic pressure when no more leaks are observed.
   With the system filled with water, pressurize it to exceed the maximum expected process operating pressure by about 10%.

Warning: Do not exceed pressure limits for the cassettes and system components or torque and hydraulic compression limits.

   Check for leaks. If liquid is found leaking from around the cassette, increase manual torque in 10% increments or the hydraulic pressure in increments of 3 bar (45 psi). Stop increasing when no more leaks are observed.

Warning: Do not exceed the maximum recommended torque or hydraulic pressure.

   If liquid is found leaking from around fittings, check the fittings and gaskets. Replace if necessary.
   Once properly installed and compressed in the holder, cassettes can be pre-conditioned and used in a process.
   A forward flow air integrity test including a system integrity (pressure hold) test should be performed prior to adding product to the system (Section : on page 27).
5. Periodically check the torque on each bolt for MT holders. Cassettes will compress initially upon installation and will require adjustment. Temperature changes to the environment or feed solution may require a torque adjust-

ment to the holder. AT holders compensate for reduced cassette compression and normally do not require periodic adjustment of the hydraulic pressure.

Caution: Increasing the temperature of the feed solution will cause expansion of the cassettes, causing the clamping force to increase unless the torque on MT assemblies or hydraulic pressure on AT assemblies is relieved or reduced. It is possible that the maximum clamping force may be exceeded. If a heated solution will be pumped into the cassette system, set the clamping force on both MT and AT holders just below the minimum setting prior to adding the heated fluid.

When finished using a calibrated torque wrench, adjust the wrench to its minimum force setting for storage. Leaving a torque wrench set to a higher value can cause it to go out of calibration.

The torque required to apply the correct clamping force on cassettes depends on the size and number of tie rods and the surface area over which the force is applied. On Pall AT hydraulic closure systems, it also depends on the diameter of the pistons. Values listed in Table 1 and Table 2 are recommended for cassettes from Pall Life Sciences. When installing cassettes from Pall Life Sciences in holders from other manufacturers, contact Pall with the specifications of the holder so that the correct torque or hydraulic pressure can be determined.

Caution: Excessive compression can permanently damage the cassette.

Table 1: Recommended Hydraulic Pressure Range for Pall AT Cassette Holders

Holder Type(1)	No. Hydraulic Pistons on Holder	Recommended Hydraulic Pressure Range for AT Holders			
		Omega™, Alpha™ and Supor® TFF Membrane Cassettes		Regen™ Membrane Cassettes	
		(psi)	(bar)	(psi)	(bar)
Centrasette LV AT	4	500 – 800	34 – 54	400 – 500	27 – 34
Centrasette 5 AT	4	500 – 800	34 – 54	400 – 500	27 – 34
Centrasette 10 AT	2	1100 – 1600	75 – 110	800 – 1000	54 – 68
Centrastak AT	2/level	1100 – 1600	75 – 110	800 – 1000	54 – 68
Maxisette AT	6	1400 – 1800	95 – 122	1100 – 1300	75 – 88

(1) Recommended hydraulic pressure ranges for these holders with Supor, Omega and Alpha cassettes have been lowered from previous versions of this guide. Changes are based on studies indicating reduced pressures will sufficiently compress cassettes in the holder.

PALL Life Sciences

Table 2: Recommended Torque Values for Pall Manual-torque Cassette Holders

Holder Type	No. Bolts on Holder	Recommended Torque Range for Manual -Torque Cassette Holders(1)			
		Omega, Alpha and Supor TFF Membrane Cassettes		Regen TFF Membrane Cassettes	
		(in-lb.)	(Nm)	(in-lb.)	(Nm)
Minisette™ (2)	4	90 – 120	10 – 14	60 – 90	7 – 10
Centramate LV	4	50 – 60	6 – 7	40 – 50	5 – 6
Centramate	4	60 – 90	7 – 10	40 – 70	5 – 8
Centrasette 5, Centrasette LV	4	300 – 450	35 – 50	150 – 300	17 – 35
Centrasette 10, Centrasette P	2	600 – 900	70 – 100	300 – 600	35 – 70
Centrasette 10	4	300 – 450	35 – 50	150 – 300	17 – 35
Maximate	4	100 – 160	11 – 8	65 – 120	7 – 14
Maxisette 25, Maxisette 50, Maxisette 100 (2)	6	700 – 1050	80 – 120	400 – 500	45 – 56

(1) Pall membrane cassettes

(2) Maxisette MT and Minisette Holders are no longer available.

3. Operating Specifications

3.1 Recommended Crossflow Rates for Pall Membrane Cassettes

Table 3 lists recommended crossflow flux (CFF) rates for operating Pall TFF membrane cassettes. Other parameters such as TMP and temperature can be evaluated at these CFF values.

CFF = L/min / ft^2 or m^2 [retentate flow rate /membrane area]

The values listed under Processing Mode are recommended for use when processing a sample. Higher flow rates are recommended for cleaning and sanitization (Cleaning Mode).

Table 3: Recommended Retentate Crossflow Flux Rates (CFF) for Pall TFF Cassettes

		Minimum CFF Processing Mode		Recommended CFF(1) Processing Mode		Recommended CFF(1) Cleaning Mode	
Holder Type	Units	Screen Channel Cassettes	Suspended Screen Channel Cassettes	Screen Channel Cassettes	Suspended Screen Channel Cassettes	Screen Channel Cassettes	Suspended Screen Channel Cassettes
Centramate or Centrasette	L/min/m^2	3	8	5 – 8	10 – 20	7 – 12	15 – 30
	L/min/ft^2	0.3	0.8	0.5 – 0.8	1.0 – 2.0	0.7 – 1.2	1.5 – 3.0
Maximate or Maxisette	L/min/m^2	2	5	4 – 5	7 – 15	6 – 10	10 – 25
	L/min/ft^2	0.2	0.5	0.4 – 0.5	0.7 – 1.5	0.6 – 1.0	1.0 – 2.5

(1) Trials must be performed to determine the most effective crossflow rate to use for any specific application.

3.2 Operating Pressures, Temperatures and pH

Operating limit specifications for pressure, temperature, and pH are listed in Table 4: Cassette Operating Limits of Pressure, Temperature, and pH.

Table 4: Cassette Operating Limits of Pressure, Temperature, and pH

			pH Range	
Membrane Type	Temperature(1)	Max. Pressure	Continuous at 25 °C (< 8 hr)	Cleaning at 45 °C (< 4 hr)
Omega Alpha	-5 to 50 °C	6.8 bar (100 psi)	1 – 14	1 – 14
Supor TFF (all formats)	4 to 25 °C	3 bar (45 psi)	1 – 14	1 – 13
	25 to 50 °C	2 bar (30 psi)	1 – 14	1 – 13
Regen	-5 to 50 °C	5 bar (75 psi)	3 – 13	3 – 13

(1) Freezing can damage cassettes. Additives such as ethanol, glycerin, or salts that depress the freezing point enable the use of cassettes at temperatures below 0 °C.

3.3 Hold-up Volume and Minimum Working Volume

It is imperative to determine the hold-up volume and minimum working volume for your system following installation of cassettes and prior to flushing out the system.

Feed/Retentate Hold-up Volume is the total volume, most of which is recoverable, contained within the feed/retentate flow path.

Minimum Working Volume is the hold-up volume plus a minimum volume of liquid that must remain in the bottom of the feed tank at the operating flow rate to prevent air from being drawn into the cassette system.

The minimum working volume limits the maximum concentration factor achievable. It is affected by the crossflow rate. At a higher crossflow rate, a greater liquid volume in the bottom of the feed tank is required to prevent air from getting drawn into the pump. Tank design significantly affects the minimum volume required to prevent air from getting into the system.

Permeate Hold-up Volume is the total volume contained within the permeate flow path

Non-Recoverable Volume is the volume remaining in the Feed/Retentate flow path after the flow channel has been pumped out and drained. Optimization of the product recovery step will ensure high product recovery.

3.3.1 Determining Feed/Retentate Hold-up Volume

This procedure assumes the system is dry and cassettes have just been installed, and that the retentate line is flexible and can be directed either to the reservoir or the drain.)

1. Add a measured volume of water into the feed reservoir that will be at least three times the expected hold-up volume for the system. Record the volume. (If insufficient volume was used, more can be added later.) Use the following table as a guide.

Table 5: Required Volumes for determining Feed/Retentate Hold-up Volume

System	Approximate volume to add(1)
Centramate	300 – 500 mL
Maximate	400 – 600 mL
Centrasette	1 – 3 L
Maxisette	5 – 15 L

(1) Actual volume will depend on tubing / piping diameter and lengths, as well as amount of membrane area installed.

Note: For bench-top systems with flexible hoses on the feed and retentate lines, add a measured volume of water to a graduated cylinder and place the ends of the feed and retentate tubing into the cylinder.

2. Close the permeate valve.
3. Open the retentate valve completely.
4. Start the pump and circulate the water through the system until no air bubbles exit the retentate line. (Adjust flow rate so feed pressure is about 0.3 bar (5 psi). If the reservoir or graduated cylinder is completely drained, add an additional measured volume of water so that residual liquid is left in the reservoir or cylinder when circulated

5a. If the liquid was initially poured into the feed reservoir, put the retentate line into a graduated cylinder, leaving the feed line in the feed reservoir, and slowly pump out the water just until the level reaches the very bottom of the reservoir. Do not allow air to be drawn into the feed line.

5b. If a graduated cylinder was initially used as the reservoir, carefully remove the feed and retentate lines from the graduated cylinder. Hold up the ends so that water doesn't run out. If the tubing is not completely filled with water, use a little water from the cylinder to fill them back up.

6. Record the volume in the graduated cylinder (B).

The difference between the starting volume, plus any volume added (A), and the volume in the graduated cylinder (B) is the feed/retentate hold-up volume (C), (Table 6: Feed/Retentate Hold-up Volume).

Table 6: Feed/Retentate Hold-up Volume

Total Volume Added	**A**	________________ mL	A
Remaining Volume In Cylinder	**B**	– ________________ mL	B
Feed/retentate Hold-up Volume	**(A - B)**	= ________________ **mL**	**C**

3.3.2 Determining Permeate Hold-up Volume

Procedure

1. Add the volume collected in the graduated cylinder to the feed reservoir (or leave in the graduated cylinder if it was used as a reservoir). If the volume collected (B) was not at least double the retentate hold-up volume (C), add an additional measured volume to the reservoir or cylinder to increase the total volume in the system to more than 3 hold-up volumes.
2. Open the retentate and permeate valves
3. Direct the feed, retentate and permeate lines into the feed reservoir or graduated cylinder.
4. Start the pump and circulate the water for a few minutes through the system until no more air bubbles are seen exiting from the retentate or permeate line. (Adjust the flow rate so feed pressure is 1– 2 bar (15 – 30 psi). Restrict the retentate valve if necessary.
5. If the liquid was initially poured into the feed reservoir, put the retentate line into a graduated cylinder, close the permeate valve, and slowly pump out the water just until the level reaches the very bottom of the reservoir.
   If a graduated cylinder was initially used as the reservoir, carefully remove the feed, retentate, and permeate lines from the graduated cylinder. Hold up the ends, so that water doesn't run out. If the tubing is not completely filled with water, use a little water from the cylinder to refill them.
6. Record the volume remaining in the cylinder (D).

The difference between the total volume added (A*) and the volume remaining in the cylinder (D) is the total system hold-up volume (feed/retentate and permeate). Subtract the feed/retentate hold-up volume (C) to get the permeate hold-up volume (F), Table 7: Total System Hold-up Volume.

Table 7: Total System Hold-up Volume

Total Volume Added	**A***[(1)]	________________ mL	A*
Remaining Volume In Graduated Cylinder	**D**	- ________________ mL	D
Total System Hold-up Volume (feed/retentate/permeate)	**(A* - D)**	= ________________mL	E
Feed/retentate Hold-up Volume (from Table 6 on page 15)	**C**	- ________________ mL	C
Permeate Hold-up Volume	**(E - C)**	= ________________ **mL**	**F**

(1) A* = initial volume of water added (A — from Table 6) plus any addition volume added from Section 3.3.2 in Step 1.

3.3.3 Determining the Non-Recoverable Volume

Procedure

1. If the liquid was initially poured into the feed reservoir, close the permeate valve, then put the retentate line into the graduated cylinder and slowly pump out the remaining water in the system (combining with the volume collected from the previous step), allowing air to purge out the lines.
   If a graduated cylinder was initially used as the reservoir, close the permeate valve, then carefully remove the feed line leaving the retentate and permeate lines in the cylinder and slowly pump out the remaining water in the system, allowing air to purge out the lines.
2. Carefully remove the permeate line from the graduated cylinder.
3. Hold the end up and fill the line with water from the cylinder.
4. Record the total volume in the graduated cylinder (G).
5. Subtract the volume in the graduated cylinder (G) plus the permeate hold-up volume (F) from the total volume added (A*).

The difference between the total volume added and the volume in the graduated cylinder plus the permeate hold-up volume is the Non-Recoverable Volume (X) (Table 8: Non-Recoverable Volume).

Table 8: Non-Recoverable Volume

Total Volume Added	**A***(2)	________________ mL	A*
Volume in Graduated Cylinder	**G**	– ________________ mL	G
Permeate Hold-up Volume	**F**	– ________________ mL	F
Non-recoverable Volume, Feed/Retentate	A*- (G + F)	= _________________**mL**	**X**

(2) A* = initial volume of water added (A — from Table 6) plus any addition volume added from Section 3.3.2 in Step 1.

3.3.4 Determining the System Minimum Working Volume

Note: For this measurement, the ***actual*** *feed reservoir must be used. This procedure may be performed after the cassettes have been sanitized.*

Procedure

1. Add a volume of water to the feed reservoir equal to at least 4 system hold-up volumes.
2. Open the retentate and permeate valves. Direct retentate and permeate lines into the feed reservoir.
3. Adjust the pump to deliver the retentate flow rate that will be used in the process and circulate the water for a few minutes through the system till no more air bubbles are seen exiting from the permeate line.
   For systems with MF membranes, adjust the permeate valve to give a flux rate in the expected range for processing. If necessary, add additional water to the reservoir to prevent air from being pulled into the feed line.
4. Direct the retentate line to drain and pump out water just until air is about to be pulled into the feed line. Then return the line to the feed reservoir. If air is pulled in, add just enough water to the reservoir, so no air gets drawn into the lines at the required flow rate.
5. Stop the pump.
6. Close the permeate valve.
7. Remove and hold the permeate line up.
8. Direct the retentate line to a graduated cylinder and slowly pump out the remaining water in the system, allowing air to purge out the lines.
9. Fill the permeate line with water from the cylinder.
10. Record the volume collected in the graduated cylinder.

To determine the Minimum Working Volume, add the Non-Recoverable volume (X) to the Volume Collected in the graduated cylinder (Y).

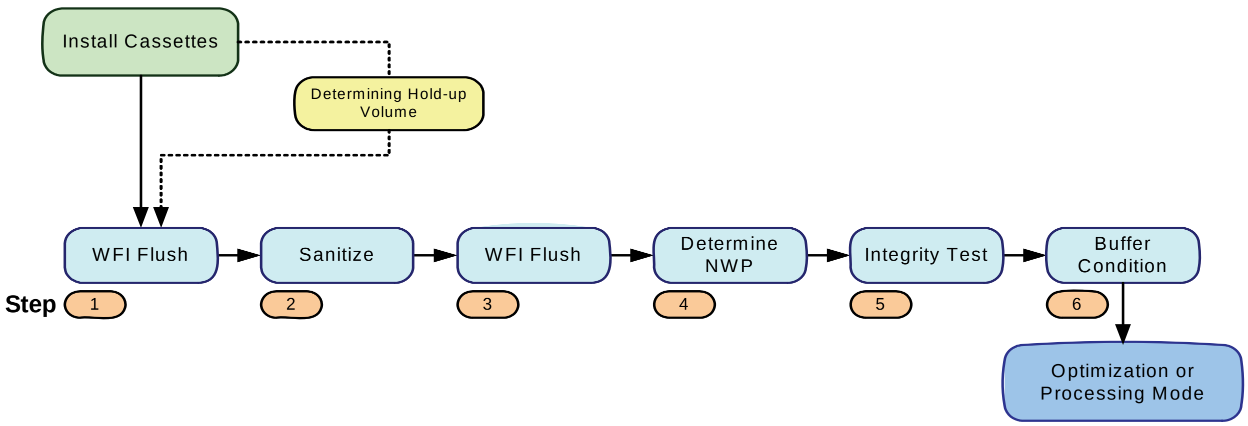
Table 9: Minimum Working Volume

Volume in Graduated Cylinder	**Y**	________________ mL	Y
Non-recoverable Volume from Table 8	**X**	+ ________________ mL	X
Minimum Working Volume	**X + Y**	**= ________________mL**	**Z**

4. Preconditioning Cassettes and System

Before processing product, several steps must be performed (Figure 8: Steps for Preconditioning TFF Cassettes) to assure that the cassettes are properly installed in the holder and fit-for-use to prevent possible sample loss or contamination from storage agents. This preconditioning process consists of six steps:

Figure 8: Steps for Preconditioning TFF Cassettes



The combination of steps 1-3 are required to assure that the storage agents have been effectively and sufficiently removed.

Step 1 WFI Flush, quickly removes the bulk of the storage agent, so only small quantities of water are required in this step.

Step 2 is designed to reduce or eliminate bioburden from the cassette and to extract most of the remaining storage agents.

Step 3 flushes out the sanitizing agent and reduces extractables to acceptable levels. Flushing volumes must be determined by the user for the specific application.

The combination of steps 4 and 5 establish the fitness of the cassette for use or reuse.

Step 4 measurement of Normalized Water Permeability (NWP) is required to establish a pre-use baseline value against which the cassette can be measured following its use in a process and subsequent cleaning. Comparing the initial NWP to the NWP value measured after cleaning is a way to establish the effectiveness of the cleaning process.

Step 5 establishes the integrity of the cassettes and system against leaks and possible product loss.

Step 6 buffer conditioning, prepares the wetted surfaces of the system as well as the membrane in the cassette before the addition of process fluid by removing trapped air from the system and equilibrating the system in the process buffer to reduce the risk of product precipitation or denaturation. It also equilibrates the system temperature to the product temperature.

Details of each step are presented in the following sections.

Warning: Exceeding the maximum operating pressure for the cassette can permanently damage the cassette.

4.1 Using a Cassette for the First Time

First time use of new membrane cassettes requires the removal of the storage agents (such as glycerin and sodium azide, or sodium hydroxide) from the cassettes. A material safety data sheet with important information about the preservative agents is included with each cassette.

The volume of flushing agent and flushing time to achieve the required minimum level of extractables may be greater for first time use of cassettes compared to subsequent use and will depend on the storage agents, membrane porosity and temperature of flushing solution.

The procedures outlined in Sections 4.2, 4.3, and 4.4 are designed to efficiently and effectively remove these agents but may need to be modified to meet your specific requirements for trace level contaminants.

4.2 Initial Flushing of the Cassette and Assembly (WFI Flush)

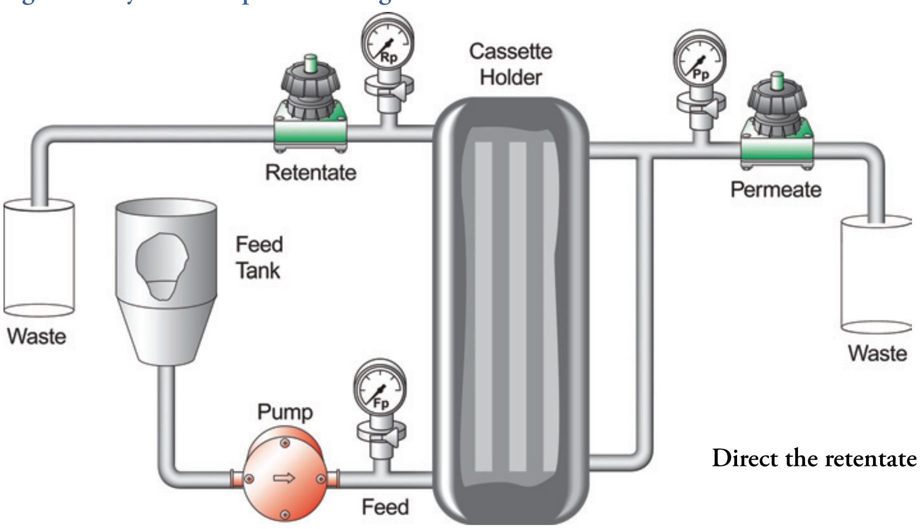
Flush the cassette and hardware assembly with pharmaceutical-grade water. Use of a lower quality water to flush the system may introduce inorganic impurities that could affect membrane performance and product recoveries.

Water Quality for flushing:

WFI (water for injection) at 25 – 45 °C or
0.2 µm filtered DI water at 25 – 45 °C
Volume Required: 10 - 40 L / m^2 (1 – 4 L/ft^2)

4.2.1 Flush the Feed/Retentate and Permeate Line to Waste

Figure 9: System Setup for Flushing



Direct the retentate and permeate line to waste.

Procedure

1. Fill the feed reservoir with water (or attach feed line to water supply).
2. Open the retentate and permeate valve.
3. Adjust the pump to deliver a flow rate of 5 – 10 L/min/m^2(0.5 – 1 L/min/ft^2).
   Do not exceed a feed pressure of 2 bar (30 psi).
   If necessary, restrict permeate valve so at least 50% of flow is out the retentate line.
4. Pass 5 – 20 L/m^2 (0.5 – 2 L/ft^2) through the retentate to waste.
   Measure the volume passing through the permeate as well as retentate.
5. Stop the pump.

4.2.2 Flush the Permeate Line to Waste

1. Close retentate valve. Open permeate valve.
2. Adjust the pump to deliver a permeate flow rate of 5 L/min/m^2 (0.5 L/min/ft^2) or until the feed pressure equals 2 bar (30 psi).

3. Pass 5 – 20 L/m^2 (0.5 – 2 L/ft^2) through the permeate.
   The permeate volume collected from the steps detailed in Section 4.2.1 can be added in considering the total volume flushed through the permeate.

4.3 Sanitizing and Depyrogenating the Cassette and Assembly

The following agents and conditions are recommended for sanitizing the specific Pall membrane types listed:

Volume Required:

5 – 20 L/m^2 (0.5 – 2 L/ft^2)

Membranes:

Omega, Alpha, and Supor TFF membranes

Sanitizing and Depyrogenating Solutions:

0.1 – 0.5 N NaOH at 25 – 45 °C

0.1 – 0.5 N NaOH + 200 – 400 ppm NaOCl at 25 – 45 °C

10 – 50 ppm NaOCl (pH 6 – 8) at 25 – 45 °C

For cassettes that may have been subjected to high levels of endotoxin, the use of an acid solution may be required as an alternative or additional procedure to the caustic processing step listed above:

0.1 N Acetic Acid (HAc) at 25 – 45 °C

0.1 N Phosphoric Acid (H_3P0_4) at 25 – 45 °C

Membrane:

Regen™

Sanitizing and Depyrogenating Solutions:

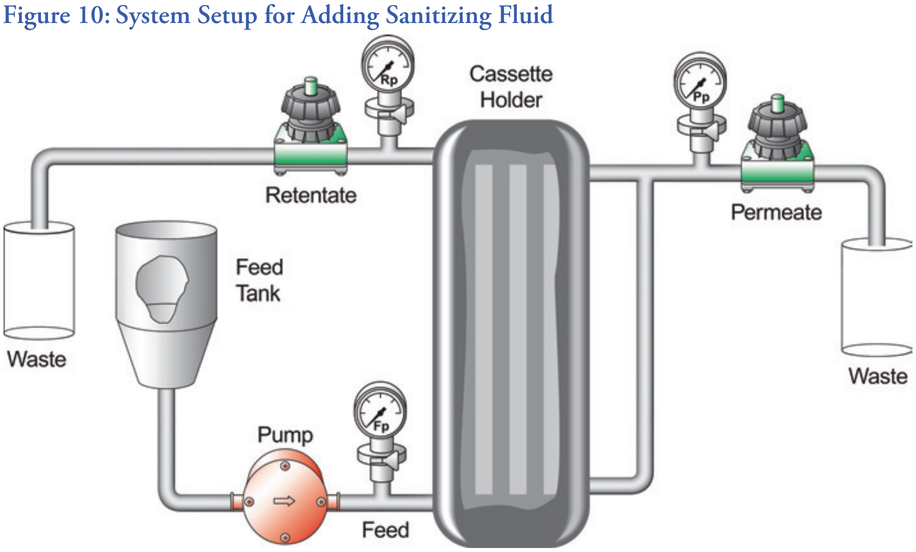
0.1 N NaOH at 25 – 45 °C

0.1 – 0.5 N HAc at 25 – 45 °C

Warning: Many solutions recommended for sanitizing and depyrogenation may be corrosive and or hazardous. Ensure proper safety procedures are followed while handling, mixing and preparing these reagents.

4.3.1 Add Sanitizing Solution

Figure 10: System Setup for Adding Sanitizing Fluid

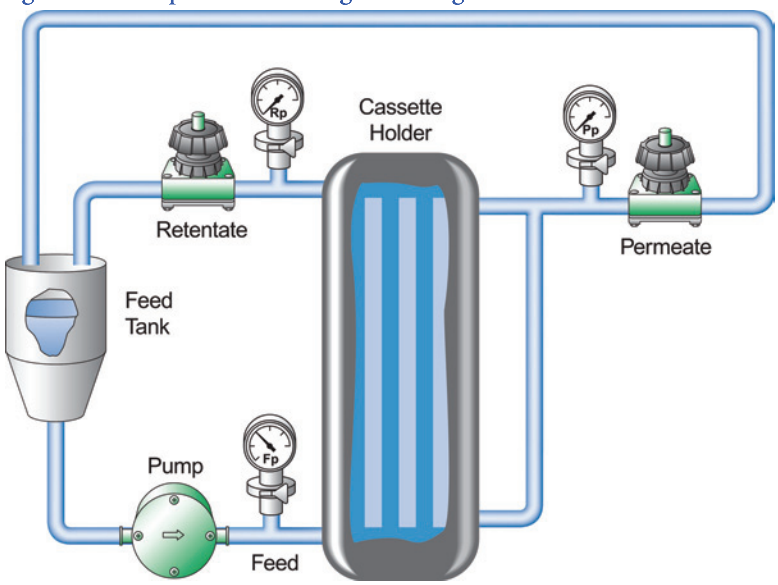


Procedure

1. Drain the system and reservoir.
2. Fill the reservoir with sanitizing solution
3. Open the permeate and retentate valves.
4. With the retentate and permeate line directed to waste, start the pump and run a small volume of sanitizing solution through the system to waste (flushing with one or two system hold-up volumes is usually sufficient). It may be necessary to partially close the retentate valve to force liquid through the permeate.
5. Stop the pump.
6. Return the retentate and permeate lines to the feed reservoir.

4.3.2 Add and Recirculate Sanitizing Solution

Figure 11: Set-up For Circulating Sanitizing Fluid



Procedure

1. For screen channel cassettes, open the retentate valve and close the permeate valve completely. Adjust the pump speed to give a retentate flow rate in the range 1 – 1.5 times the recommended process CFF rate in Table 3. Do not exceed a feed pressure of 2.8 bar (40 psi).
   For suspended screen channel cassettes, keep the permeate valve open. Then adjust the pump speed to give a retentate flow rate in the range 1 – 1.5 times the recommended process CFF rate in Table 3. If the permeate flow rate is significantly greater than the retentate flow rate, reduce the pump speed, then adjust the permeate valve until the retentate and permeate flow rates are approximately equal, then readjust the retentate flow rate.

With suspended screen cassettes, the permeate pressure should not exceed the retentate pressure. If necessary, restrict the retentate valve to maintain a positive pressure at the retentate and adjust other parameters as required to achieve desired results.

2. Run for 30 – 60 minutes.

The effectiveness of this sanitization procedure to reduce or eliminate bioburden will depend on the nature and level of the contamination. The user must evaluate and validate the effectiveness of the process with respect to time and temperature and the effectiveness of the sanitizing agent for their process.
With process systems, it may be necessary to keep the permeate valve open with screen channel cassettes to assure effective sanitization. Process systems often have multiple flow paths that must be flushed with sanitizing solution in the proper sequence in order to assure effective sanitization. It is up to the user to determine the appropriate procedure for their system.

4.4 Flushing the Cassette and Assembly after Sanitization

Water Quality:

WFI at 25 – 45 °C or

0.2 µm filtered DI water at 25 – 45 °C

Volume Required

40 L/ m^2 (4 L/ft^2) minimum

The actual volume of water required will depend on a number of factors including:

Whether the product is in concentrate or permeate — If the product is in the permeate, a greater volume of water will be required to reduce the permeate TOC (total organic carbon) to acceptable levels compared to the retentate.

Membrane type and pore size — Small pore UF membranes (30 kDa) require significantly greater volumes than MF and open pore UF membranes

Flushing water temperature — Increased temperature reduces required volume.

Retentate / Permeate flow rates — Lower flow rates reduce required volume

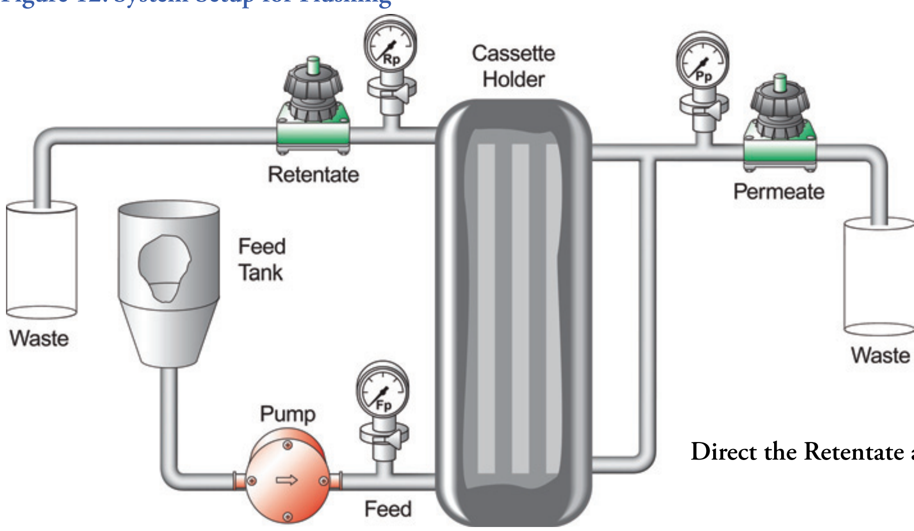
Storage agent — High viscosity agents will extract slower

Minimum acceptable level of extractables and method of detection — such as TOC (total organic carbon), NVR (non-volatile residue), UV (ultraviolet adsorption), conductivity.

A flushing study may be required to determine the appropriate volumes to use for your application.

4.4.1 Flush the Retentate and Permeate Line to Waste

Figure 12: System Setup for Flushing



Direct the Retentate and Permeate lines to Waste

Procedure

1. Drain, wash and refill feed reservoir with water (or attach feed line to water supply).
2. Open the retentate and permeate valve.
3. Adjust the pump to deliver a flow rate of 5 – 10 L/min/m^2(0.5 – 1 L/min/ft^2). Do not exceed a feed pressure of 2 bar (30 psi). If necessary, restrict permeate valve so at least 50% of flow is out the retentate line.
4. Pass 10 – 20 L/m^2 (1 – 2 L/ft^2) through the retentate to waste.
5. Stop the pump

4.4.2 Flush the Permeate Line to Waste

Procedure

1. Close the retentate valve. Open the permeate valve
2. Adjust the pump to deliver a permeate flow rate of 5 L/min/m^2 (0.5 L/min/ft^2) or until the feed pressure equals 2 bar (30 psi).
3. Open the retentate valve until the retentate flow rate is 5 – 10% of the permeate flow rate.
4. Run until a minimum of 20 L/m^2 (2 L/ft^2) is flushed through the permeate or until the pH and/or TOC has reached an acceptable value — normally close or equal to the pH/TOC of the incoming water.

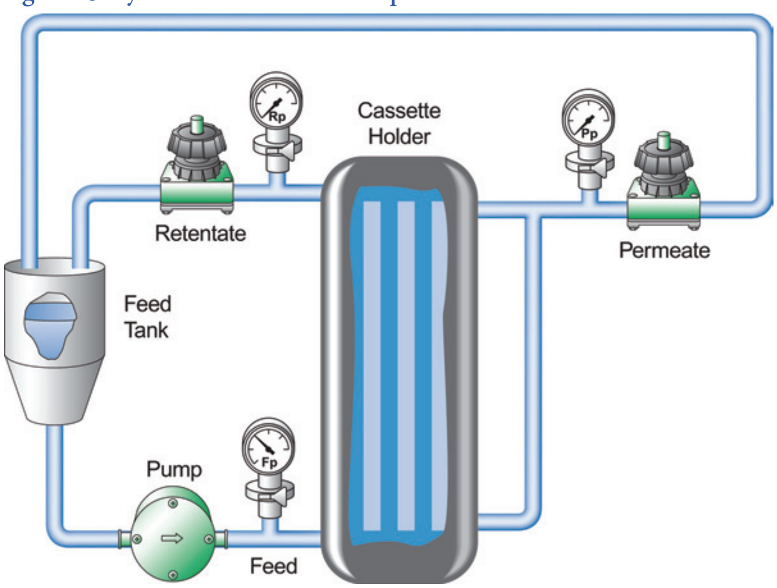
The previous flushing procedure is effective in removing sanitizing solution and remaining extractables from the system, but may require a significant quantity of water.
The addition of one or more recirculation steps through both retentate and permeate can reduce the total volume of water needed to achieve the required pH, TOC or extractable levels. A typical recirculation procedure is described in Section 4.4.3: Recirculation Procedure to Reduce pH, TOC and Extractables (optional) on page 23.

4.4.3 Recirculation Procedure to Reduce pH, TOC and Extractables (optional)

Procedure

1. Set up the system for flushing to waste (Figure 9: System Setup for Flushing on page 19).
2. Perform steps 4.4.1 and 4.4.2 using minimum volumes of water for initial flush.
3. Set up the system for recirculation.

Figure 13: System Recirculation Setup



4. Add 5 – 10 L/m^2 (0.5 – 1 L/ft^2) to reservoir (a smaller volume/ft^2 is acceptable for larger systems).
5. Open the retentate and permeate valves.
6. Adjust the pump to deliver a flow rate in the processing range for the type of cassette used (Table 3: Recommended Retentate Crossflow Flux Rates (CFF) for Pall TFF Cassettes on page 13).
7. Adjust the retentate valve (or permeate valve), so that the permeate flow rate is approximately equal to the retentate flow rate. Do not exceed a feed pressure of 2 bar (30 psi). Readjust the pump speed to deliver the recommended retentate flow rate or until the feed pressure reaches 2 bar (30 psi).
8. Circulate fluid for 30 – 60 minutes.
9. Perform steps 4.4.1 and 4.4.2 using minimum volumes of water for flush. Take samples of retentate and permeate from the effluent at end of the flush and assay. If pH, TOC, extractables levels are not acceptable, repeat steps 3 to 9 until acceptable levels have been obtained.

4.5 Determine Normalized Water Permeability ($NWP_{20\,°C}$) for Cassettes

The initial $NWP_{20\,°C}$ of the membrane cassette is essential to calculate because it is used as the basis for determining membrane recovery (i.e., how effectively the membrane was cleaned back to its original state).

This procedure must be performed with all new cassettes after steps 4.2 through 4.4 are completed.

Water quality should be *Water for Injection* (WFI) or minimally 0.2 µm filtered DI water.

All calculated water permeability rates are normalized to a temperature of 20 °C by applying a temperature correction factor ($TCF_{20\,°C}$) given in Table 11: Temperature Correction Factors (TCF) for Normalizing Water Permeability.

The procedure recommended for performing NWP is with the retentate valve closed (Dead-end Method). This will apply a uniform pressure profile across the pathlength of the cassette.

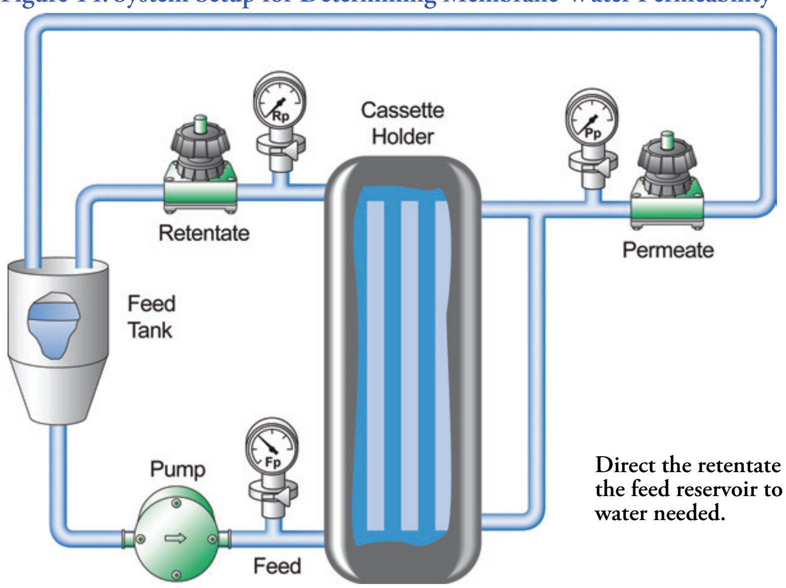
NWP should be measured on the cassette after sanitization/flushing (Section 4.3: Sanitizing and Depyrogenating the Cassette and Assembly on page 20) and then again after cleaning and flushing (Section 5.3: Flush Cleaning Agent from Cassettes and Assembly on page 37). An example for determining NWP is shown in Section 4.5.4: An Example of How to Determine Membrane Water Permeability on page 26.

> *Note: If the cleaning protocol for the process uses harsher conditions (such as higher concentration, higher temperature, or a different cleaning agent) than the sanitization procedure, then it is recommended that a full cleaning protocol and flushing be performed prior to performing the initial $NWP_{20\,°C}$.*

4.5.1 Remove Air from Cassette and System

> *Caution: It is important to completely remove air from the feed channels to wet out the membrane before determining $NWP_{20\,°C}$. Both permeate ports should be open for determining $NWP_{20\,°C}$. Closing one port can cause a significant error in TMP measurement due to internal pressure drops, especially with UF membranes > 100 kDa or MF membranes.*

Figure 14: System Setup for Determining Membrane Water Permeability



Direct the retentate and permeate lines back to the feed reservoir to minimize the volume of water needed.

Procedure

1. Check the water level in the reservoir and refill if necessary.
2. Open the permeate and retentate valve completely.
3. Adjust the pump speed to deliver the recommended crossflow flux. Refer to Table 3: Recommended Retentate Crossflow Flux Rates (CFF) for Pall TFF Cassettes on page 13.
   (i) With MF, suspended screen channel cassettes, it may be necessary to restrict the permeate valve to achieve the desired retentate flow rate.

   (ii) If required, set the pump flow rate to the required retentate flow rate.
   (iii) Adjust the permeate valve till retentate and permeate flow rates are approximately equal.
   (iv) Then readjust the pump speed to deliver the required retentate flow rate.
4. Close the retentate valve till the feed pressure increases by 0.7 – 1 bar (10 – 15 psi) or the valve is completely closed, then open immediately.

Caution: Do not exceed a feed pressure of 3 bar (45 psi).

5. Repeat previous step at least three times to remove air.

4.5.2 Determine Initial Water Permeability (Dead-end Method)

Procedure

1. Close the retentate valve and open the permeate valve.
2. Adjust the feed pump flow rate to generate the desired transmembrane pressure (TMP).
   Start at the lowest TMP (Transmembrane Pressure).

Equation 3: Calculation for Transmembrane Pressure

$$TMP = \left(\frac{P_{feed} + P_{retentate}}{2}\right) - P_{permeate}$$

It is recommended that water permeability be measured at three different TMP values.
• up to 1 bar (15 psi) for UF membranes ≤ 100 kDa and
• up to 0.65 bar (10 psi) for UF membranes over 100 kDa and for MF membranes
For example, choose the pressures 0.3, 0.65, 1.0 bar (5, 10, 15 psi) for a 10 kDa membrane cassette. Choose the highest TMP, so that the permeate flow rate does not exceed 150 LMH

3. Quickly open and then close the retentate valve at least three times to expel any trapped air.
4. Measure the permeate flow rate and calculate the permeate flux rate in units of LMH ($L/m^2/hr$).
5. Measure the temperature of the permeate stream.
6. Adjust the feed flow rate to the next transmembrane pressure and repeat steps 2 – 5.
7. Repeat steps 2 – 5 at the highest TMP.
8. Plot the water permeate flux rate vs. TMP for the 3 points measured.
9. Draw a straight line from the origin that best fits the data points plotted.
   Note: The plot should be linear for UF membranes. If the points do not fall close to the line, check the pressure gauges for accuracy and re-measure.

The accuracy and reproducibility of measurements are very dependent on the accuracy and readability of the pressure gauges or transducers used. Be sure to use calibrated equipment that is accurate within the measurement range.

10. From the graph, determine the water permeate flux rate at the transmembrane pressure in Table 10: Measuring Water Permeability for the membrane type used.

Table 10: Measuring Water Permeability

Membrane	MWCO or Pore Size	TMP
UF	0.65 kDa – 100 kDa	0.7 bar (10 psi)
UF	>100 kDa	0.3 bar (5 psi)
MF	0.1 µm – 1.2 µm	0.1 – 0.2 bar (2 psi)

Use pressure gauges and transmitters with a maximum range of 0 – 2 bar (0 – 30 psi) for accurate, repeatable results.

With some MF membrane cassettes, it may not be possible to achieve a TMP above 0.2 bar (3 psi). In this case take a single measurement at the highest achievable TMP (Transmembrane Pressure)).

11. Calculate the water permeability at the recorded TMP from Equation 4: Water Permeability Calculation.

Equation 4: Water Permeability Calculation

$$WaterPermeability = \frac{PermeateFluxRate(LMH)}{TMP}$$

4.5.3 Normalize the Water Permeability

1. Normalize the water permeability to a temperature of 20 °C using Equation 5: Normalized Water Permeability Calculation and the temperature correction factors ($TCF_{20\,°C}$) in Table 11: Temperature Correction Factors (TCF) for Normalizing Water Permeability on page 27.

Equation 5: Normalized Water Permeability Calculation

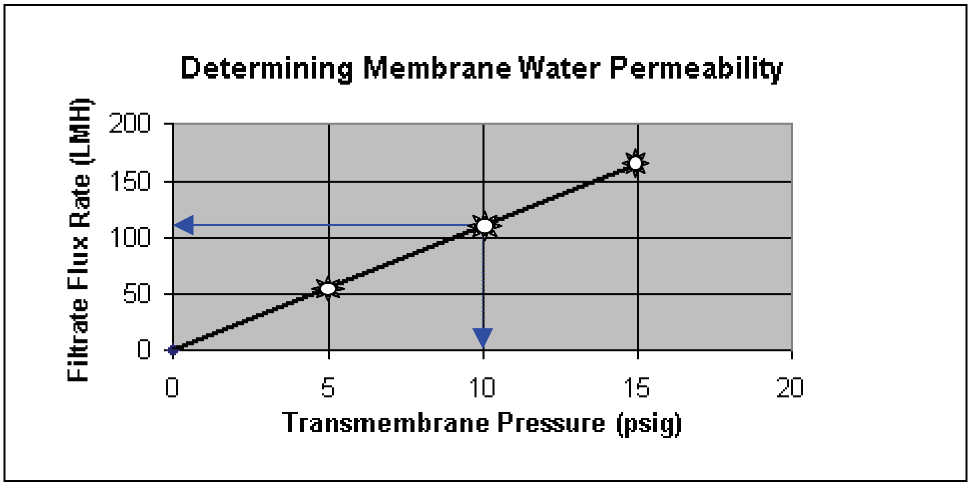
$$NWP_{20°C} = \frac{PermeateFluxRate(LMH)}{TMP} \times TCF_{20°C}$$

4.5.4 An Example of How to Determine Membrane Water Permeability

Example: Water permeate flux rates were measured for a 10 kDa UF membrane cassette at transmembrane pressures of 5, 10, and 15 psi and plotted in Figure 15: Determining Membrane Water Permeability. The temperature of the water was 16 °C.

Determine the initial normalized water permeability ($NWP_{20\,°C}$).

Figure 15: Determining Membrane Water Permeability



Results:

Water Permeability = 110 LMH @ 10 psi = 11.0 LMH/psi

Normalized Water Permeability = 11.0 LMH/psi x $TCF_{20\ °C}$ where $TCF_{20\ °C}$ = 1.109

(Correcting the water temperature from 16 °C to 20 °C)

Normalized Water Permeability ($NWP_{20\ °C}$) = 11.0 x 1.109 = 12.2 LMH/psi

Table 11: Temperature Correction Factors (TCF) for Normalizing Water Permeability

Temperature Correction Factors ($TCF_{20\ °C}$)									
T °C	$TCF_{20\ °C}$	T °C	$TCF_{20\ °C}$	T°C	$TCF_{20\ °C}$	T °C	$TCF_{20\ °C}$	T°C	$TCF_{20\ °C}$
		11	1.27	21	0.98	31	0.78	41	0.64
		12	1.24	22	0.95	32	0.76	42	0.63
		13	1.20	23	0.93	33	0.75	43	0.62
4	1.57	14	1.17	24	0.91	34	0.73	44	0.61
5	1.52	15	1.14	25	0.89	35	0.72	45	0.60
6	1.47	16	1.11	26	0.87	36	0.70	46	0.59
7	1.43	17	1.08	27	0.85	37	0.69	47	0.58
8	1.39	18	1.05	28	0.83	38	0.68	48	0.57
9	1.35	19	1.03	29	0.81	39	0.66	49	0.56
10	1.31	20	1.00	30	0.80	40	0.65	50	0.55

4.6 System and Membrane Cassette Integrity Test

Air diffusion (or forward flow) is a quantitative test that measures the rate of air diffusing through the wetted membrane or seal defects at a given pressure differential. Since the measurements are relative, air diffusion rates can be performed on cassettes wetted with water or buffer solution.

Before performing a forward flow air integrity test on a membrane cassette, the membrane must be completely wetted out. Sections 4.2 through 4.4 should be performed before performing the integrity test.

If the system temperature will be altered from the current value, it is recommended that buffer conditioning and temperature stabilization (Section 4.7) should be performed *before* the integrity test.

Refer to Figure 16: System Setup for Determining Air Integrity for the setup with the Palltronic Flow Star Integrity Analyzer. This instrument regulates air and pressure to perform the forward flow air integrity test. The Palltronic Flow Star Integrity Analyzer measures and displays the rate of air that is diffusing through the membrane.

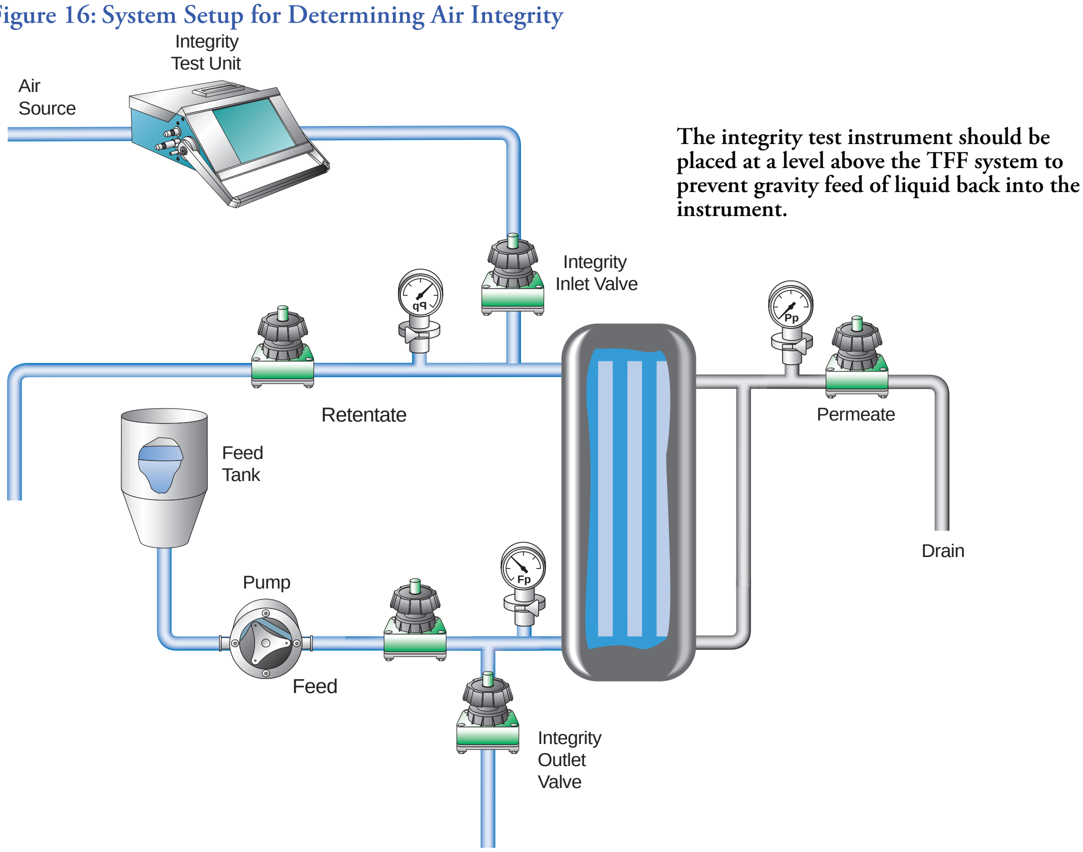
There are two important steps to the integrity test protocols:

1. **System Integrity Test** (external test)
   The **System Integrity Test** checks the external seals, fittings, plumbing and gasket assemblies within the pressurized feed /retentate flow path for leaks.
2. **Membrane Cassette Integrity Test** (internal test)
   The **Membrane Cassette Integrity Test** checks the membrane and internal seal assemblies within the cassette for defects.

The **System Integrity Test** is usually performed first. This assures that any airflow measured during the **Membrane Cassette Integrity Test** is not the result of an external leak.

Performing a System Integrity Test is recommended but not required. If the Membrane Cassette Integrity Test passes, the integrity of the membranes and system is confirmed. However, if the **Membrane Cassette Integrity Test** fails, then the **System Integrity Test** should be performed to confirm that no external leaks contribute to the measured airflow.

Figure 16: System Setup for Determining Air Integrity



4.6.1 Drain the System (Feed / Retentate Flow Path)

Note: The feed/retentate flow path must be drained of liquid before performing the system or membrane cassette integrity test.

The procedure for draining the feed retentate flow path will depend on the system configuration. The objective is to remove most of the liquid. It is not necessary to completely remove all liquid.

Procedure

The liquid can be removed by any combination of the following steps:

1. Opening the drain and vent ports on the system to allow liquid to drain out
2. Draining or disconnecting the reservoir and then using the feed pump (positive displacement) to pump air into the system to displace liquid out through the retentate line
3. Using an integrity test air source to pressurize with air to force liquid out through feed/retentate line. Liquid can also be forced out through the membrane and permeate line

4.6.2 System Integrity Test (External Test)

Procedure

1. Connect the integrity test unit to the system.

The integrity tester may be connected as shown in Figure 15: System Setup for Determining Air Integrity, or you can disconnect the pump and attach the air supply from the integrity analyzer in its place. The integrity tester may also be connected to the retentate line if an isolation valve is placed between the feed port and pump.

2. Close the permeate valve and any other valves in the feed and retentate flow path as required to allow the system to be pressurized with air.
3. Charge the system to a pressure of 2 bar (30 psi).
4. The airflow rate displayed on the integrity test unit will increase at first, then drop off. As the pressure stabilizes to 2 bar (30 psi), the air mass flow rate should decrease to zero (as shown in the example given in Figure 17: Typical Mass Air Flow Example Plotted for System and Membrane Integrity Test on page 31.
   If the mass flow rate = zero (< 5 sccm for membrane area > $0.1 m^2$ [1 ft^2]), the system integrity has passed, so continue with step 5.
   If the mass flow rate levels off above zero (> 5 sccm), go to Section 4.6.4 "Troubleshooting System Integrity Failure" on page 30.

On some systems, it may take 15 minutes or longer for airflow to drop to zero, since the air diffuses through the membrane to pressurize the permeate side. If there is still airflow (> 5 sccm) after the permeate and feed/retentate pressures are equal, a leak is indicated.

5. Once the system pressure test has been completed, slowly open the permeate valve to relieve the pressure.
6. Slowly open the integrity outlet valve or the retentate valve to reduce the air pressure to zero in the feed/retentate channels.
7. Close the integrity outlet valve or retentate valve.

4.6.3 Membrane Cassette Integrity Test

Membrane Type: Omega, Alpha, Supor TFF, Regen

Cassette Format: Centramate, Centrasette, Maximate, Maxisette

Channel Format: Screen, Suspended screen, Open

Procedure

1. Set the pressure on the integrity test unit to the value listed in Table 12: Pressure and Airflow Specifications for Pall Membrane Cassettes.
2. Open the permeate valve.

Table 12: Pressure and Airflow Specifications for Pall Membrane Cassettes

Membrane MWCO or Pore Size	Test Pressure	Allowable air Forward Flow Rate per unit area of membrane
500 kDa and lower MWCO	2.0 bar (30 psi)	≤ 1600 sccm/m^2 (≤ 150 sccm/ft^2)
1000 kDa and 0.16 µm Omega	0.7 bar (10 psi)	≤ 1076 sccm/m^2 (≤ 100 sccm/ft^2)
Supor TFF, 0.1 – 0.8 µm	0.7 bar (10 psi)	≤ 270 sccm/m^2 (≤ 25 sccm/ft^2)

3. Turn on the air supply from the integrity tester to pressurize the system.
4. The air flow rate displayed on the integrity test unit will initially increase as the system pressurizes, then drop before levelling off to a constant flow rate. This measured constant mass flow rate is the air forward flow (diffusion) rate (Figure 17: Typical Mass Air Flow Example Plotted for System and Membrane Integrity Test on page 31).
5. If the constant mass flow rate falls below the acceptance specification listed in Table 12 on page 30 (and is consistent with previous measurements), the membrane integrity has passed.

If the mass flow rate is above the recommended specification or substantially above previous measurements, go to Section 4.6.5: Troubleshooting Membrane Integrity Failure on page 30.

4.6.4 Troubleshooting System Integrity Failure

Procedure

1. If a manual-torque (MT) holder is used, check the torque.
   If an auto-torque (AT) assembly is used, check to make sure the hydraulic pressure is at the correct setting.
2. Check all sanitary clamp connections and fitting assemblies to assure they are securely tightened.
3. Remove the cassettes and rinse with WFI water. Inspect the cassette gaskets and the holder contact surfaces for imperfections. If the gaskets look worn or deformed, install new gaskets. Reinstall the cassettes in the holder.
4. Repeat the System Integrity Test, Section 4.6.2: System Integrity Test (External Test) on page 29.

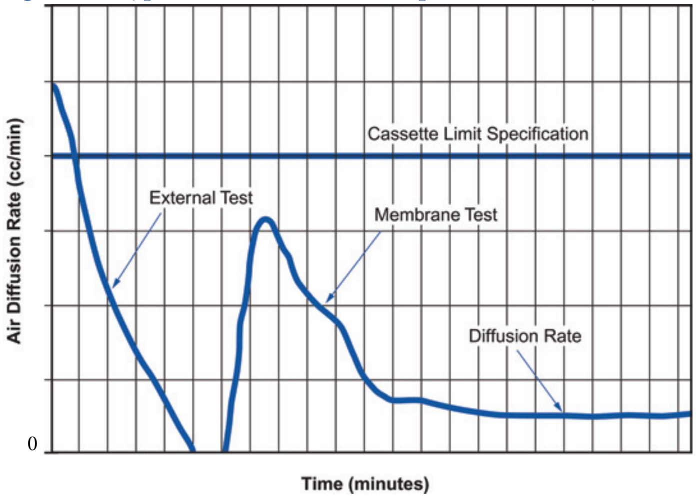
4.6.5 Troubleshooting Membrane Integrity Failure

Procedure

1. If a manual-torque (MT) holder is used, check the torque.
   If an auto-torque (AT) assembly is used, check to make sure the hydraulic pressure is set correctly.
2. Repeat the System Integrity Test.
3. Remove the cassettes and rinse with WFI. Inspect the cassette gaskets for imperfections.
   If the gaskets look worn or deformed, install new gaskets. Reinstall the cassettes in the holder.
4. Repeat the Membrane Cassette Integrity Test (Section 4.6.3: Membrane Cassette Integrity Test on page 29).
   If the cassette still fails an integrity test, contact your local Pall representative for assistance.

If multiple cassettes are installed and the air diffusion rate continues to exceed specification limits, disassemble the stack of cassettes and test the integrity of each cassette individually to determine if integrity failure is specific to a cassette.

Figure 17: Typical Mass Air Flow Example Plotted for System and Membrane Integrity Test



External Test

As the system fills with air to the targeted test pressure, the air flow rate will decrease to zero (base line). If it does not reach zero, the system has an external leak. Check all fittings and valves.

Internal Membrane Test

When the permeate valve is open, the air flow rate will rise. Then the flow rate will decrease to a steady state, which is the true air diffusion (forward flow) rate.

4.6.6 Integrity Testing Systems with Multiple Cassettes

True integrity can only be validated on individual cassettes. Hence, testing should first be done on each cassette separately. Then the individual cassettes can be installed in the holder and tested as a unit. For a stack of cassettes, the air diffusion rate is additive and should approximate the sum of the individual cassettes.

4.7 Buffer Conditioning

Buffer conditioning the membrane cassettes and TFF assembly is critical for most biopharmaceutical applications.

Buffer conditioning removes trapped air from the membrane cassette and system and establishes conditions for pH, ionic strength and temperature to match the process sample. This is important to prevent sample precipitation or denaturation when it is first introduced into the system.

For this operation, use the same buffer that was used to prepare the sample.

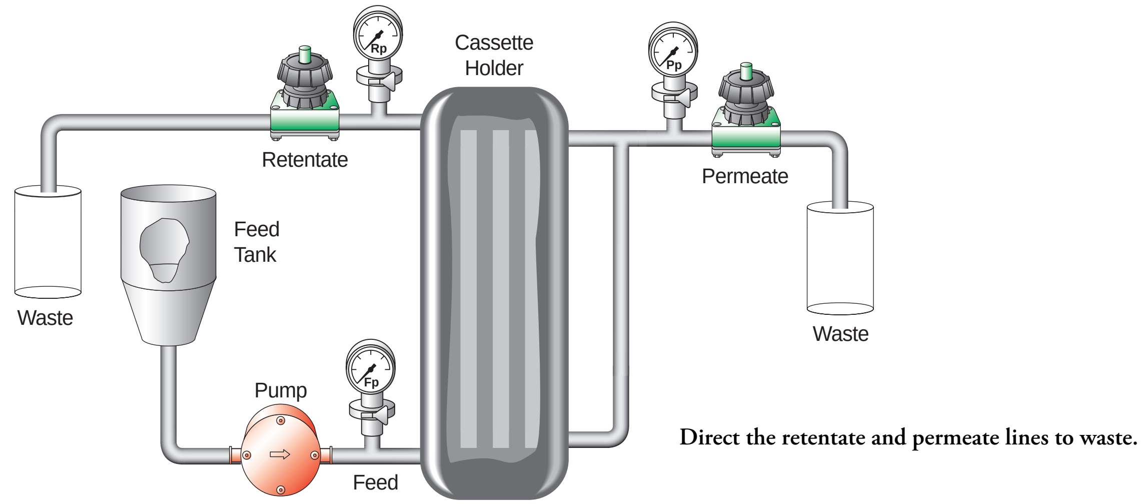
The buffer must be at the same temperature as the process fluid.

Membrane cassettes and hardware that are subject to substantial temperature changes (> 10 °C) will require a torque adjustment to be made on the cassette holder after steady state is reached. For example, if the hardware surface temperature is 20 – 25 °C and fluid is introduced at 4 °C, the torque on a full stack of cassettes may drop 20 –40% due to contraction of the polymeric elements. In such cases, integrity testing should be performed after all adjustments have been made.

Volume of Buffer Required: 5 – 20 L/m^2 (0.5 – 2 L/ft^2). (Less buffer may be required for large process systems.)

4.7.1 Flush the Retentate and Permeate Lines with Buffer

Figure 18: Setup for Buffer Conditioning — Initial Flush

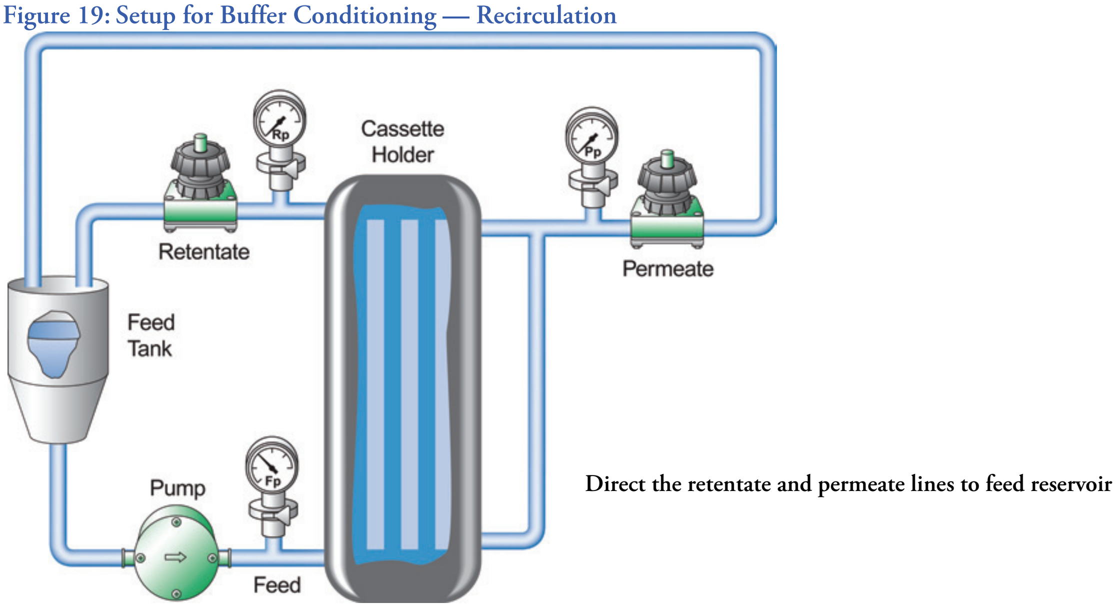


Procedure

1. Drain the system and feed reservoir, then fill feed reservoir with buffer.
2. Open the permeate and retentate valves.
3. Start the pump and increase pump speed until liquid begins to flow through both the retentate and permeate lines. If necessary, adjust the retentate valve to force flow through permeate.
4. Run for 5 – 10 seconds, then stop.

4.7.2 Remove Trapped Air from the Retentate Line

Figure 19: Setup for Buffer Conditioning — Recirculation



Procedure

1. Set up the system for recirculation.
2. Open the retentate and permeate valves.
3. Adjust the pump speed to give the recommended crossflow flux. Refer to Table 3: Recommended Retentate Crossflow Flux Rates (CFF) for Pall TFF Cassettes on page 13.

With MF, suspended screen channel cassettes, it may be necessary to restrict the permeate valve to achieve the desired retentate flow rate. If required, set the pump flow rate to the required retentate flow rate. Adjust the permeate valve until the retentate and permeate flow rates are approximately equal. Then readjust the pump speed to give the required retentate flow rate.

4. Close the retentate valve *until* the feed pressure increases by 0.7 – 1 bar (10 – 15 psi) or valve is completely closed, then open immediately.

Caution: Do not exceed a feed pressure of 3 bar (45 psi).

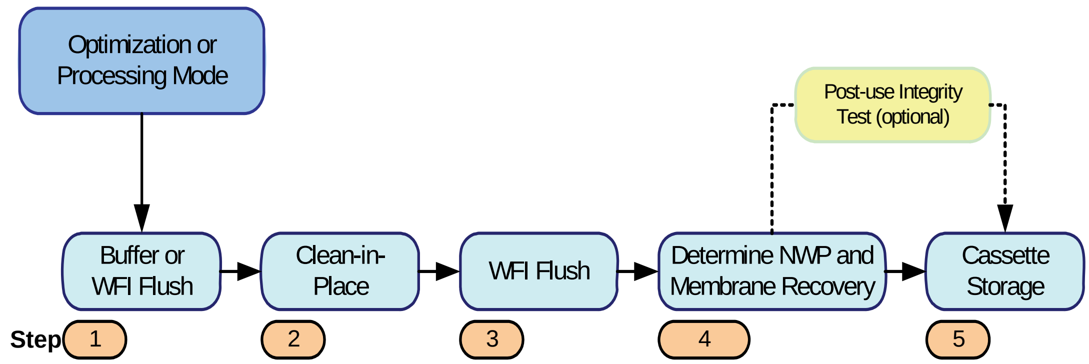
5. Repeat this step at least three times to remove air.
6. If necessary, adjust the pump flow rate to maintain a feed pressure of 1 – 2 bar (15 – 30 psi) for UF membranes and 0.2 – 0.7 bar (3 – 10 psi) for MF membranes.
7. Recirculate fluid for 10 – 15 minutes if conditioning for pH and ionic stability at ambient temperature. For equilibrating temperature between the hardware and fluid stream, continue until the temperature has stabilized.
8. Check the torque on the cassette holder (not required for auto-torque (AT) systems).
9. Remove the excess buffer from the feed vessel. Keep all piping, tubing, and cassette assembly to and from the feed vessel flooded with buffer to prevent air from entering the system.

It is necessary to determine the system hold-up volume in the feed/retentate flow path. This volume will dilute the process fluid and must be considered when calculating concentration factor. Refer to Section 3.3: Hold-up Volume and Minimum Working Volume on page 14.

5. Post-use Treatment of Cassettes and System

Once the TFF Process has been completed and the product recovered, the membrane cassettes can be cleaned and reused. There are several steps that must be followed to remove remaining contaminants and return the cassettes to initial condition.

Figure 20: Sequence of Steps for Post-use Treatment of TFF Cassettes



The cleaning process should also be documented and validated if cassettes will be reused in production of a biopharmaceutical product. This flushing and cleaning process takes considerable time and uses substantial quantities of water and cleaning agent. It may be worth evaluating the economics of cleaning and validation for reuse compared to discarding cassettes after a single use.

5.1 Flushing Cassettes after Use

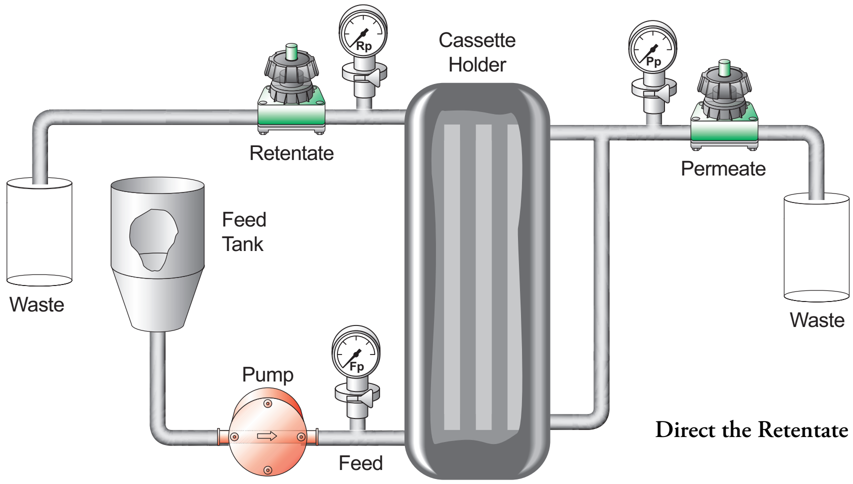
Following a TFF process, the product, if in the concentrate, would have likely been recovered by displacement with buffer followed by a recirculation and flushing with a small additional volume of buffer. In this case, it may not be necessary to flush the cassette before adding cleaning solution.

If the product was in the permeate, the concentrate should be flushed from the cassette using water or buffer prior to introducing cleaning solution.

5.1.1 Flush the Feed / Retentate Line to Waste

Volume Required 10 L/m^2 (1.0 L/ft^2)

Figure 21: System Setup for Flushing to Waste



1. Drain and refill the feed reservoir with 10 – 40 L/m^2(1 – 4 L/ft^2) buffer or WFI.
2. Open the retentate and permeate valves.
3. Adjust the pump to deliver a flow rate of 5 – 10 L/min/m^2 (0.5 – 1 L/min/ft^2). Do not exceed a feed pressure of 2 bar (30 psi). If necessary, restrict the permeate valve, so at least 50% of flow is removed from the retentate line.
4. Pass a minimum of 5 – 20 L/m^2 (0.5 – 2 L/ft^2) through the retentate line to waste. Measure the volume passing through the permeate as well as the retentate.
5. Stop the pump.

5.1.2 Flush the Permeate Line to Waste

1. Close the retentate valve. Open the permeate valve.
2. Adjust the pump to deliver a permeate flow rate of 5 L/min/m^2 (0.5 L/min/ft^2) or until the feed pressure equals 2 bar (30 psi).
3. Pass 5 – 20 L/m^2(0.5 – 2 L/ft^2) through the permeate. The volume collected in step 5.1.1 can be added in considering the total volume flushed through the permeate.

5.2 Cleaning Cassettes

Volume Required	5 L/m^2 (0.5 L/ft^2) minimum

Recommended cleaning agents for Pall membranes
(see Section 6.2: Alternative Cleaning Agents on page 46 for alternatives):

Membrane Types	Omega, Alpha, Supor TFF
Cleaning Solutions	0.1 – 0.5 N NaOH at 25 – 45 °C
	0.1 – 0.5 N NaOH + 200 – 400 ppm NaOCl at 25 – 45 °C
Membrane Type	Regen
Cleaning Solution	0.1 N NaOH at 25 – 45 °C

Warning: Many solutions that are recommended for cleaning may be corrosive. Ensure proper safety procedures are followed while handling, mixing and preparing these reagents.

5.2.1 Adding Cleaning Agent to System

1. Drain the system and reservoir.
2. Fill the reservoir with 5 – 20 L/m^2 (0.5 – 2 L/ft^2) of cleaning solution.
3. Open the permeate and retentate valves.

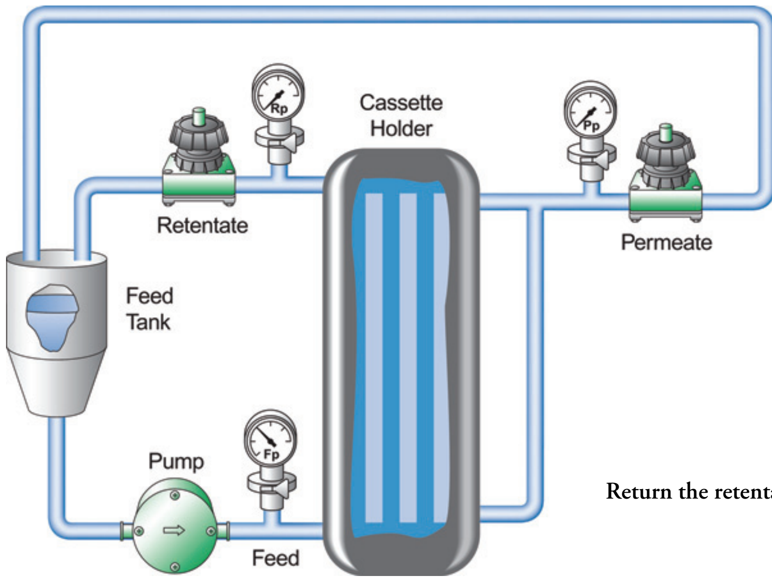
4. With the retentate and permeate line still directed to waste, start the pump and run a small volume of cleaning solution through the system to waste.

Typically flushing with one or two system hold-up volumes is sufficient. It may be necessary to partially close the retentate valve to force liquid through the permeate.

5. Stop the pump.
6. Return the retentate and permeate line back to the feed reservoir.

5.2.2 Recirculate Cleaning Solution

Figure 22: System Setup for Circulating Cleaning Solution



Return the retentate line to the feed reservoir.

1a. For screen channel cassettes, open the retentate valve and close the permeate valve completely. Adjust the pump speed to give a retentate flow rate in the range of 1 – 1.5 times the recommended process CFF rate in Table 3: Recommended Retentate Crossflow Flux Rates (CFF) for Pall TFF Cassettes on page 13.

Caution: Do not exceed a feed pressure of 2.8 bar (20 – 40 psi).

1b. For suspended screen channel cassettes, keep the permeate valve open. Adjust the pump speed to give a retentate flow rate in the range 1 – 1.5 times the recommended process CFF rate in Table 3: Recommended Retentate Crossflow Flux Rates (CFF) for Pall TFF Cassettes on page 13.
If the permeate flow rate is significantly greater than the retentate flow rate, reduce the pump speed, then adjust the permeate valve until the retentate and permeate flow rates are approximately equal, then readjust the retentate flow rate.
With suspended screen cassettes, the permeate pressure should not exceed the retentate pressure. If necessary, restrict the retentate valve to maintain a positive pressure at the retentate and adjust other parameters as required to achieve desired results.

2. Run 45 – 60 minutes for cleaning.

If the cleaning solution looks dirty after only a few minutes, stop cleaning and drain the cleaning solution. Follow steps 5.1.1 and 5.1.2 to flush with water through the retentate than the permeate. Add fresh cleaning solution to the feed reservoir and follow the steps in Section 5.2.1: Adding Cleaning Agent to System on page 35.

5.3 Flush Cleaning Agent from Cassettes and Assembly

Flush the cassette and hardware assembly with either:

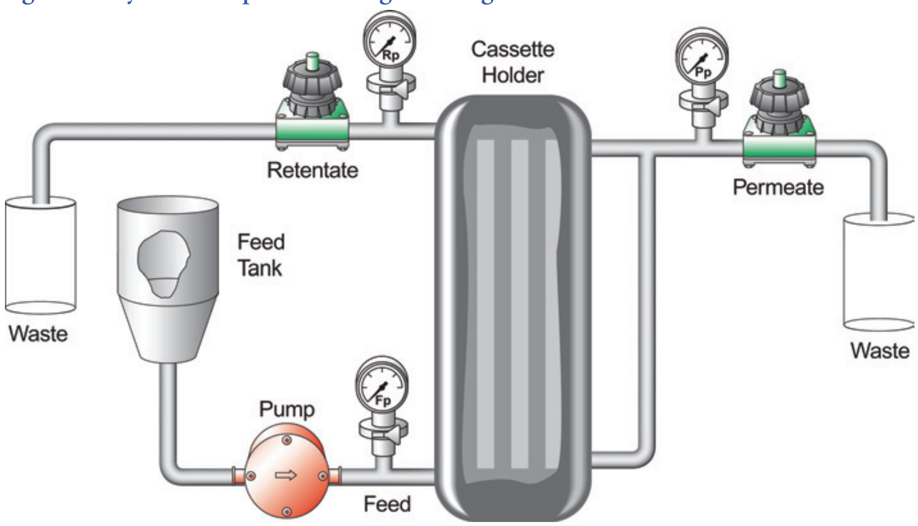
1. WFI at 25 – 45°C, or
2. 0.2 µm filtered DI water at 25 – 45 °C

Volume required: 40 L/m^2 (4 L/ft^2) minimum

To establish required flushing volumes for a specific application, test the pH and/or conductivity of water from the retentate to determine when acceptable conditions have been reached. If the product will be in the permeate, test the pH and conductivity of the permeate.

5.3.1 Flush the Retentate Line to Waste

Figure 23: System Setup for Flushing Cleaning Solution to Waste



1. Drain, wash, and refill the feed reservoir with water (or attach the feed line to the water supply).
2. Open the retentate and permeate valve.
3. Adjust the pump to deliver a flow rate of 5 – 10 L/min/m^2 (0.5 – 1 L/min/ft^2). Do not exceed a feed pressure of 2 bar (30 psi). If necessary restrict the permeate valve, so at least 50% of flow is from the retentate line.
4. Pass 10 – 20 L/m^2 (1 – 2 L/ft^2) through the retentate to waste.
5. Stop the pump.

5.3.2 Flush the Permeate Line to Waste

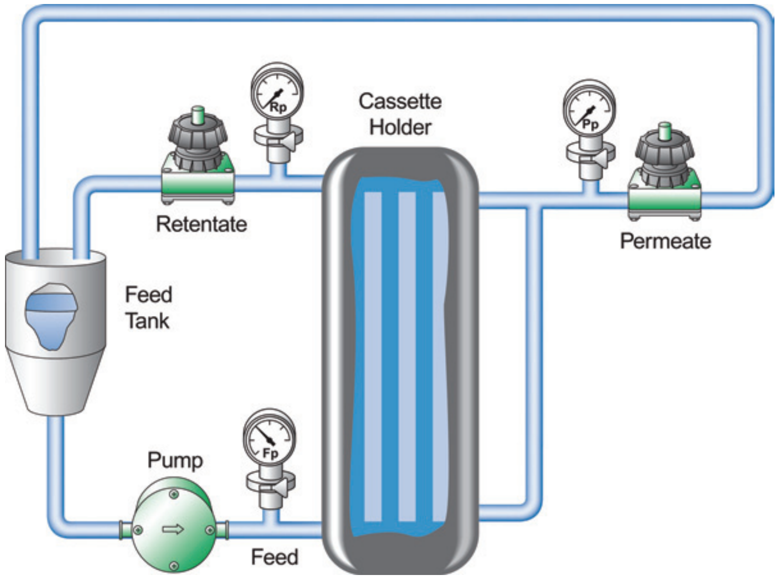
1. Close the retentate valve.
2. Open the permeate valve
3. Adjust the pump to deliver a permeate flow rate of 5 L/min/m^2 (0.5 L/min/ft^2) or until the feed pressure equals 2 bar (30 psi).
4. Open the retentate valve until the retentate flow rate is 5 – 10% of the permeate flow rate.
5. Run until a minimum of 20 L/m^2 (2 L/ft^2) is flushed through the permeate, or until the pH and/or TOC has reached an acceptable value — normally close or equal to the pH/TOC of the incoming water.

The addition of one or more recirculation steps through both retentate and permeate can be used to reduce the total volume of water needed to achieve the required pH, TOC, or extractable levels. See Section 5.3.3: Recirculation Procedure to Reduce pH, TOC, and Extractables (optional) on page 37.

5.3.3 Recirculation Procedure to Reduce pH, TOC, and Extractables (optional)

1. Set up the system for flushing to waste (Figure 23: System Setup for Flushing Cleaning Solution to Waste)
2. Perform the steps in sections 5.3.1 and 5.3.2 using minimum volumes of water for initial flush.
3. Set up the system for recirculation (Figure 24: System Setup for Recirculation).

Figure 24: System Setup for Recirculation



4. Add 5 – 10 L/m^2 (0.5 – 1.0 L/ft^2) to reservoir (A smaller volume/ft^2 is acceptable for larger systems)
5. Open the retentate and permeate valves.
6. Adjust the pump to deliver a flow rate in the processing range for the cassette type used (Table 3: Recommended Retentate Crossflow Flux Rates (CFF) for Pall TFF Cassettes on page 13).
7. Adjust retentate valve (or permeate valve) so permeate flow rate is approximately equal to retentate flow rate. Do not exceed a feed pressure of 2 bar (30 psi). Readjust pump speed to give recommended retentate flow rate or feed pressure reaches 2 bar (30 psi).
8. Circulate liquid for 30 – 60 minutes.
9. Perform steps 5.3.1 and 5.3.2 using minimum volumes of water for flush. Take samples of retentate and permeate from the effluent at end of flush and assay. If the pH, TOC, and extractable levels are not acceptable, repeat steps 3 to 9 until acceptable levels have been obtained.

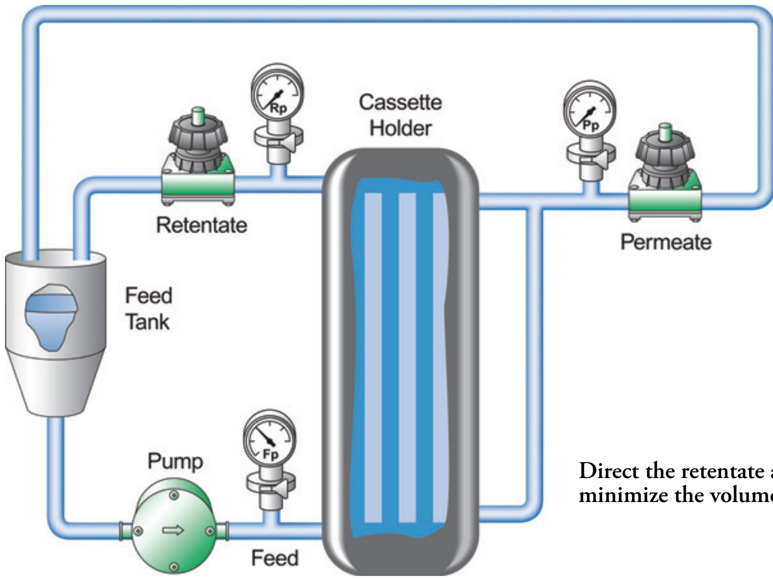
5.4 Determine Membrane Recovery for the Cassettes

Membrane recovery is a calculation to determine the efficiency of the cleaning performed on the membranes. It compares the normalized water permeability (NWP) after cleaning to the initial NWP, measured when the membrane was first installed and preconditioned (Section 4.5: Determine Normalized Water Permeability (NWP20 °C) for Cassettes on page 24).

Water quality should be Water for Injection (WFI) or, at minimum, 0.2 µm filtered DI water. All water permeability rates are normalized to a temperature of 20 °C by using a temperature correction factor ($TCF_{20\ °C}$) obtained from Table 11: Temperature Correction Factors (TCF) for Normalizing Water Permeability on page 27.

5.4.1 Remove Air from Cassette and System

Caution: It is important to completely remove air from the feed channels and wet out the membrane before determining $NWP_{20\ °C}$.



Direct the retentate and permeate lines back to the feed reservoir to minimize the volume of water needed.

Figure 25: System Setup For Determining Membrane Water Permeability

Caution: Both permeate ports should be open for determining $NWP_{20\,°C}$. Closing one port can cause a significant error in TMP measurement due to internal pressure drops, especially with UF membranes > 100 kDa or MF membranes.

1. Check the water level in reservoir and refill if necessary.
2. Open the permeate and retentate valve completely.
3. Adjust the pump speed to deliver the recommended crossflow flux. Refer to Table 3: Recommended Retentate Crossflow Flux Rates (CFF) for Pall TFF Cassettes on page 13.
   With MF, suspended screen channel cassettes, it may be necessary to restrict the permeate valve to achieve the desired retentate flow rate.
   (i) If required, set the pump flow rate to the required retentate flow rate.
   (ii) Adjust the permeate valve until the retentate and permeate flow rates are approximately equal.
   (iii) Then readjust the pump speed to give required retentate flow rate.
4. Close the retentate valve till the feed pressure increases by 0.7 – 1 bar (10 – 15 psi) or the valve is completely closed, then open immediately.

Caution: Do not exceed a feed pressure of 3 bar (45 psi).

5. Repeat the previous step at least three times to remove air.

5.4.2 Determine the Water Permeability after Cleaning

Procedure

1. Close the retentate valve and open the permeate valve.
2. Adjust the feed pump flow rate to generate the desired transmembrane pressure (TMP).
   Start at the lowest TMP (Transmembrane Pressure).

Equation 6: Calculation for Transmembrane Pressure

$$TMP = \left(\frac{P_{feed} + P_{retentate}}{2}\right) - P_{permeate}$$

It is recommended that water permeability be measured at three different TMP values.
• up to 1 bar (15 psi) for UF membranes ≤ 100 kDa and
• up to 0.65 bar (10 psi) for UF membranes over 100 kDa and for MF membranes
For example, choose the pressures 0.3, 0.65, 1.0 bar (5, 10, 15 psi) for a 10 kDa membrane cassette. Choose the highest TMP, so that the permeate flow rate does not exceed 150 LMH

3. Quickly open and then close the retentate valve at least three times to expel any trapped air.
4. Measure the permeate flow rate and calculate the permeate flux rate in units of LMH ($L/m^2/hr$).
5. Measure the temperature of the permeate stream.
6. Adjust the feed flow rate to the next transmembrane pressure and repeat steps 2 – 5.
7. Repeat steps 2 – 5 at the highest TMP.
8. Plot the water permeate flux rate vs. TMP for the 3 points measured.
9. Draw a straight line from the origin that best fits the data points plotted.

   Note: The plot should be linear for UF membranes. If the points do not fall close to the line, check the pressure gauges for accuracy and re-measure.

The accuracy and reproducibility of measurements are very dependent on the accuracy and readability of the pressure gauges or transducers used. Be sure to use calibrated equipment that is accurate within the measurement range.

10. From the graph, determine the water permeate flux rate at the transmembrane pressure in Table 13: Measuring Water Permeability for the membrane type used.

Table 13: Measuring Water Permeability

Membrane	MWCO or Pore Size	TMP
UF	0.65 kDa – 100 kDa	0.7 bar (10 psi)
UF	>100 kDa	0.3 bar (5 psi)
MF	0.1 µm – 1.2 µm	0.1 – 0.2 bar (2 psi)

Use pressure gauges and transmitters with a maximum range of 0 – 2 bar (0 – 30 psi) for accurate, repeatable results.

With some MF membrane cassettes, it may not be possible to achieve a TMP above 0.2 bar (3 psi). In this case take a single measurement at the highest achievable TMP (Transmembrane Pressure)).

11. Calculate the water permeability at the recorded TMP from Equation 7: Water Permeability Calculation.

Equation 7: Water Permeability Calculation

$$WaterPermeability = \frac{PermeateFluxRate(LMH)}{TMP}$$

5.4.3 Normalize the Water Permeability

Normalize the water permeability to a temperature of 20 °C using Equation 8: Calculation for Normalized Water Permeability and the temperature correction factor ($TCF_{20\,°C}$) in Table 11: Temperature Correction Factors (TCF) for Normalizing Water Permeability on page 27.

Equation 8: Calculation for Normalized Water Permeability

$$NWP_{20°C} = \frac{PermeateFluxRate(LMH)}{TMP} \times TCF_{20°C}$$

5.4.4 Determine the Membrane Recovery

Calculate the membrane recovery using Equation 9: Calculation for Membrane Recovery.

Equation 9: Calculation for Membrane Recovery

$$MembraneRecovery = \frac{NWP_{aftercleaning}}{NWP_{initial}} \times 100\%$$

5.4.5 Evaluate the Effectiveness of the Cleaning Regimen

For a new process, if membrane recovery is less than 100%, the cleaning regimen should be repeated. This is to determine that the initial cleaning time at the selected conditions was sufficient, and if not, to determine the appropriate number of cleaning cycles or time required. If membrane recovery has improved after the second cleaning cycle, a third cycle should be performed. When no additional improvement in recovery is achieved, you know the last cycle time had reached maximum effect for the conditions used. You may then use one cleaning cycle for the established time or two or more cycles of shorter duration to equal the desired time. Several cycles may be preferred, especially if cleaning is done at an elevated temperature and the system cannot maintain temperature of the cleaning fluid.

Studies altering conditions, cycle time, temperature, or reagent concentration should be performed to evaluate the most effective cleaning protocol for your process

Typically, if the membrane recovery is less than 80% after establishing the appropriate cleaning cycle time, then the cleaning agent or process parameters (such as the temperature, and crossflow rate) may have to be altered.

The effectiveness of the cleaning regimen should be evaluated over at least three process cycles. It is not uncommon to find an initial drop in membrane recovery (NWP) of 10 – 15% or more after the first process cycle. However, after this initial drop, membrane recovery should be fairly consistent from cycle to cycle. A continual decrease in recovery indicates that the cleaning regimen may not be effective and should be re-evaluated.

Note: Between uses, membrane cassettes are typically stored in a caustic solution (0.05 – 0.1 N NaOH). During this time, remaining foulants on the membrane may be degraded and removed when the membrane cassettes are flushed prior to the next use. As a result of membrane relaxation and additional cleaning during storage, it is not unusual for the membrane recovery, measured after storage, to increase relative to the membrane recovery measured immediately after cleaning.

5.5 Post-Use Air Integrity Test (optional)

Post-use air integrity tests are not usually performed on TFF cassettes. There are no requirements, as with sterilizing grade filters, that the cassette must pass a post-use integrity test. Usually analysis of results, including performing a mass balance to establish if there is product loss and where the loss might be, is sufficient to establish that the system is working satisfactorily. If you wish to determine post-use integrity, follow the procedure given in Section : on page 27.

5.6 Storage of Membrane Cassettes

The objective for proper storage of the membrane cassettes is to ensure the membranes remain wet and prevent microbial growth during the time the membrane cassettes are not being used.

Compression on cassettes stored in the holder should be reduced during storage. Reduce the torque or hydraulic pressure by 25%. Check that there is no external leaking from around the cassettes.

5.6.1 Recommended Storage Agents for the Pall Membranes

Recommended storage agents for Pall membranes

Table 14: Storage Agents for Omega, Alpha, and Supor TFF Membranes

Time	Solution
< 6 months	0.05 – 0.1 N NaOH (1)
> 6 months	15% glycerin + 0.05% sodium azide

(1) Cassettes may be stored for periods up to 1 year or longer. However, it is recommended that at 6 months storage, the pH of the storage solution be tested to determine if it is sufficiently caustic. Cassettes may be flushed with fresh storage solution at this time.

Table 15: Storage Agents for Regen Membranes

Time	Solution
< 1 month	0.05 N NaOH
> 1 month	15% glycerin + 0.05% sodium azide

Table 16: Recommended Storage Temperatures for Used Cassettes

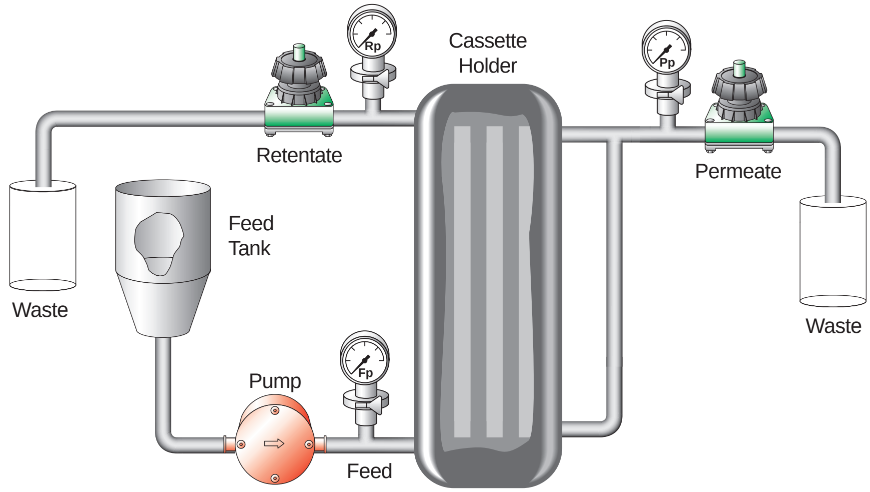
Temperature	
4 – 15 °C	optimal
25 °C	maximum

Caution: Do not freeze.

If cassettes will be stored in water, skip Sections 5.6.2 and 5.6.3 go to Section 5.6.4: Cassette Storage on page 44.

5.6.2 Adding Storage Agent to System

Figure 26: System Setup for Flushing Storage Agent through System



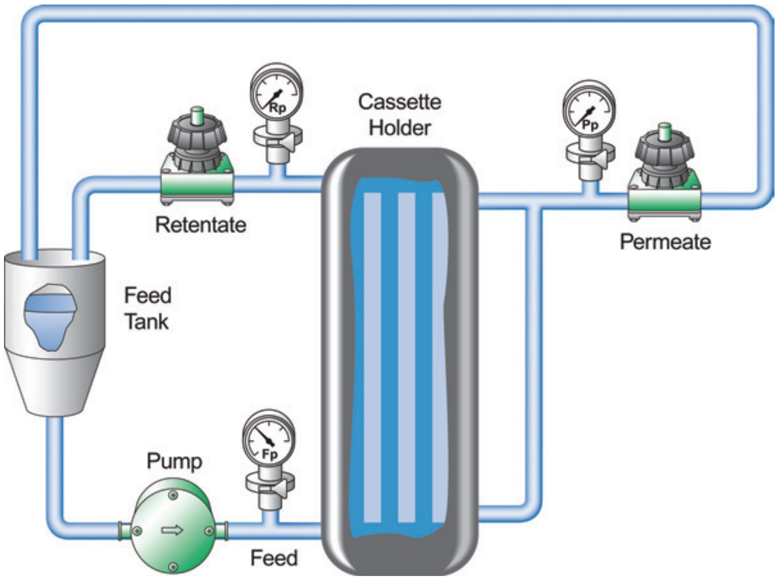
Volume of Storage Agent. Required 5 – 10 L/m^2 (0.5 – 1 L/ft^2)

Procedure

1. Drain the system and add storage agent to the feed reservoir.
2. Open the retentate and permeate valves.
3. Start the pump and increase speed until the flow comes through both the retentate and permeate lines. Allow a volume equivalent to about 2 holdup volumes to pass out of the retentate to waste
4. Allow a volume equivalent to about 2 holdup volumes to flow from the permeate to waste.
   If necessary, partially close the retentate valve to increase permeate flow rate.

5.6.3 Recirculate Storage Solution

Figure 27: System Setup for Recirculating Storage Solution



Procedure

1. Open the retentate valve. Leave permeate valve partially open
2. Adjust the pump speed to maintain a feed pressure of 1 – 2 bar (15 – 30 psi) for UF membranes and 0.2 – 0.7 bar (3 – 10 psi) for MF membranes.
3. Adjust permeate valve so that the permeate flow rate is 5 – 10% of feed flow rate within the specified pressure range.
4. Run for 3 – 5 minutes.
5. Stop the pump.

5.6.4 Cassette Storage

Procedure for Storage in the Holder

1. Close the feed, retentate, and permeate valves. (The holder and cassettes remain flooded with storage agent.)
2. Reduce the torque or hydraulic pressure on the holder by 25%.
3. Check that there are no external leaks from around the cassettes.
4. Increase the torque or hydraulic pressure slightly if it is necessary to stop a leak.

Procedure for Storage out of Holder

1. Place the cassette into a plastic bag and seal. Use a separate bag for each cassette.
   A small amount of storage agent may be added to the bag.
2. Place the bag with the cassette into a plastic container with a lid.
3. Keep the cassettes in a temperature-controlled room, optimally at a temperature of 4 – 15 °C.

Alternatively

1. Place the cassettes in a plastic container with an airtight seal.
2. Add storage agent to cover the cassettes completely.
3. Cover and seal the container.
4. Keep the container in a temperature-controlled room, optimally at a temperature of 4 – 5 °C.

6. Appendix

6.1 Membrane Chemical Compatibility Chart

The chemical compatibility of membrane cassettes can be described in terms of changes in physical characteristics due to continuous contact with a chemical solution for several hours. Changes can affect dimensions, hardness, swelling, the integrity of internal seals, and membrane integrity. Changes can also be described in terms of the functional characteristics of the membrane (such as water permeability, and retention characteristics).

Table 17 illustrates the compatibility of different membrane cassettes at 20 °C, unless otherwise noted, with respect to physical characteristics. The table should only be used as a guide. Cassettes should be tested in the appropriate solvent and product under actual operating conditions for an appropriate time to determine compatibility for a specific application.

Table 17: Membrane Chemical Compatibility Chart (1)

Compatible ✔ Not Compatible ✘

	Membrane			
Reagent	**Omega**	**Alpha**	**Supor TFF**	**Regen**
pH Range	1 – 14	2 – 13	1 – 14	3 – 13
Acetic Acid (5%)	✔	✔	✔	✔
Alconox* (1%)	✔	✔	✔	✔
Ammonium hydroxide (5%)	✘	✘	✘	✘
Citric acid (1%)	✔	✔	✔	✔
Ethanol (70%)	✔	✔	✔	✔
Formaldehyde (1%)	✔	✔	✔	✔
Glycerine (50%)	✔	✔	✔	✔
Guanidine HCl (6 M)	✔	✔	✔	✔
Hydrochloric acid (0.1 N)	✔	✔	✔	✔
Hydrogen peroxide (1%)	✔	✔	✔	✔
Phosphoric acid (0.1 N)	✔	✔	✔	✔
Sodium dodecyl sulfate (0.01M)	✔	✔	✔	✔
Sodium hydroxide (0.5 N @ 50 °C)	✔	✔	✔	✘
Sodium hypochlorite (0.05%)	✔	✔	✔	✘
Terg-a-zyme* (1%)	✔	✔	✔	✔
Triton* X-100 (0.002 M)	✔	✔	✔	✔
Urea (25%)	✔	✔	✔	✔

(1) May cause changes in porosity and/or selectivity of membrane.
** Alconox and Terg-a-zyme are trademarks of Alconox, Inc.*
** Triton is a trademark of Dow Chemical Company.*

Pall offers validation services to test compatibility of membranes and cassettes with different solvents. Contact your local Pall representative for information on Validation Services.

6.2 Alternative Cleaning Agents

Table 18: Alternative Cleaning Agents

Agent	Mode of Cleaning	Foulant
Alkalies	High pH hydrolysis Solubilization	Biomolecules, fats, proteins, starches
Acids	Solubilization	Inorganic Salts
Oxidizers	Oxidation Degradation	Biomolecules, proteins, polysaccharides
Surfactants	Wetting, emulsification, dispersion, solubilization	Biomolecules, fats, oils, proteins, insoluble particles
Solvents	Solubilization	Oils, fats, grease, proteins, biomolecules
Enzymes	Enzymatic digestion	Proteins

Table 19: Acids

Type	Foulant	Condition
Nitric (HNO_3)	Mineral Scale	0.1 N, 25 – 45 °C, pH 1
Phosphoric (H_3PO_4)	Inorganic, Nucleic Acids	0.1 N, 25 – 45 °C. pH ~2
Citric Acid	Iron	1%, 25 – 45 °C, pH ~3

Table 20: Alkalies and Oxidizers

Type	Foulant	Condition
Sodium Hydroxide (NaOH)	Proteins, enzymes, vaccines, viruses, bacterial cells and lysates, polysaccharides, organic colloids, pyrogens, lipids	0.1 – 0.5 N, 25 – 45 °C, pH >13
Sodium Hydroxide with Sodium Hypochlorite (NaOCl)	Heavy fouling of above foulants, bacterial whole cells and cell lysates	0.3 – 0.5 N NaOH + 200 – 400 ppm NaOCl, 25 – 45 °C, pH >12

Table 21: Surfactants

Type	Foulant	Condition
Sodium Dodecyl Sulfate (SDS), Triton X-100, Alconox*, Terg-a-zyme***	Bacterial whole cells and lysate, lipids, oils, antifoams, polysaccharides, and precipitated proteins	0.1%, 25 – 45 °C, pH 4 – 9

** Alconox and Terg-a-zyme are trademarks of Alconox, Inc.*
***Triton is a trademark of Dow Chemical Company.*

7. Glossary

Air Diffusion Rate	Method to determine membrane cassette integrity. Also called Forward Flow Value. It is the rate of gas diffusing through the wetted pores, or bypassing seals of the membrane at a given differential pressure.
Concentrate (Retentate)	The feed solution remaining above the membrane during or after concentration.
CIP (Clean in Place)	Act of using a chemical cleaning protocol to clean the membrane and membrane assembly free of any foulants.
Crossflow Rate (RFR) (Retentate Flow Rate)	The flow of fluid across (parallel to) the membrane surface measured as L/min.
Crossflow Flux Rate (CFF)	The crossflow rate (RFR) per unit area of membrane surface. Measured as $L/min/m^2$ or $L/min/ft^2$.
Depyrogenate	The removal or inactivation of pyrogens (endotoxins, lipopolysaccharides) from within the membrane cassette. Pyrogens are substances that cause a fever reaction when injected into mammals.
Endotoxin	The outer cell wall of gram-negative bacteria.
Feed	The starting sample volume.
Feed Pressure	The pressure (bar/psi) measured directly at the feed port (inlet) to the cassette holder.
Filtrate (Permeate)	The portion of sample (feed solution) that has passed through the membrane.
Filtrate Flow Rate (Permeate flow rate)	The rate of sample flow through the membrane (rate of sample filtration), measured in volume/unit time.
Filtrate Flux Rate (Permeate Flux Rate)	The rate of sample flow through a given membrane area per unit time. Commonly expressed as LMH ($L/m^2/hr$).
Fouling	A build-up of retained or adsorbed species on the membrane surface resulting in decreased flux rate and possibly an increase in rejection of permeable solutes.
Foulant	The material that coats, adsorbs to, or plugs pores in the membrane causing a reduction in flow rate through the membrane. The foulant may be the product or impurities (organic or inorganic) in the product or from other sources like water, buffers, etc.
Hold-up Volume	The total volume contained within the feed/retentate flow path. (Most of this volume is recoverable.)
Membrane Recovery	The percent ratio of the water NWP (normalized water permeability) after cleaning to the primary NWP measured before the membrane came into contact with a process fluid.

$$MembraneRecovery = \frac{NWP_{aftercleaning}}{NWP_{initial}} \times 100\%$$

Microfiltration (MF)	Membranes with pore sizes greater than 0.1 microns (µm) through 10 microns. Used in TFF mode for clarification or removal of micro particulates such as bacteria, mycoplasma, large virus, aggregates, whole cells and cellular debris from a solution.
Minimum Working Volume	The (system) hold-up volume plus a minimum volume of liquid that must remain in the bottom of the feed tank at the operating flow rate to prevent air from being drawn into the cassette system. The minimum working volume limits the maximum concentration factor achievable. It is affected by crossflow rate. At a higher crossflow rate, a greater liquid volume in the bottom of the feed tank is required to prevent air from getting drawn into the pump. Tank design significantly affects the minimum volume required to prevent air from getting into the system.
Molecular Weight Cutoff (MWCO)	The pore size designation for ultrafiltration membranes. The MWCO is defined as the molecular weight of a globular protein or solute which is 90% retained by that membrane after a 2X concentration. Rejection of molecules of the same molecular weight, but which have elongated shapes, can be significantly lower. The gel layer, which forms during ultrafiltration, can alter rejection characteristics of the membrane.
Non-Recoverable Volume	The volume remaining in the Feed/Retentate flow path after the flow channel has been pumped out/drained. Optimization of the product recovery step will insure high product recovery.
Normalized Water Permeability (NWP)	The water flux rate for a given membrane measured at 20 °C, divided by the average applied pressure (TMP).

$$NWP_{20°C} = \frac{PermeateFluxRate(LMH)}{TMP} \times TCF_{20°C}$$

NVR	Non-volatile residue
Permeate (Filtrate)	The portion of sample (feed solution) that has passed through the membrane.

Permeate Flow Rate (Filtrate Flow Rate)	The rate of sample flow through the membrane (rate of sample filtration), measured in volume/unit time.
Permeate Flux Rate (Filtrate Flux Rate)	The rate of sample flow through a given membrane area per unit time. Commonly expressed as LMH (liters/m^2/hr).
Permeate Hold-up Volume	The total volume contained within the permeate flow path.
Pyrogen	A substance that produces a fever within a warm-blooded animal when injected into the bloodstream.
Retentate	The liquid that flows through the feed / retentate channels of the cassette, returning from the cassette (retentate port) back to the feed reservoir.
SIP	Steam in Place. Process where steam sterilization is used in a complete assembled system.
Screen Channel	A tangential flow cassette that contains a screen in the feed/retentate channel. The screen generates resistance to flow (P) and a gentle turbulence at the membrane surface to minimize the formation of a gel layer.
System Hold-up Volume (feed/retentate)	The total volume contained within the feed/retentate flow path. (Most of this volume is recoverable.)
Tangential Flow Filtration (TFF) (Crossflow Filtration)	A process which uses a pump to circulate sample across the top of membranes ("tangential" to the membrane surface) housed in a multilevel structure (cassette). The pressure resulting from the crossflow through the narrow channels acts as the driving force to transport solute and small molecules through the membrane. The crossflow of liquid over the membrane surface sweeps retaining molecules from the surface, keeping them in the circulation stream.
TOC	Total Organic Carbon
Transmembrane Pressure (TMP)	The differential pressure from the upstream side of the membrane (applied pressure) and downstream side (permeate). The applied pressure is the average between the feed and retentate pressure.

$$TMP = \left(\frac{P_{feed} + P_{retentate}}{2}\right) - P_{permeate}$$

Ultrafiltration (UF)	A filtration technique that uses controlled pore, semi permeable membranes to concentrate or fractionate dissolved molecules. Molecules much larger than the pores are retained in the feed solution and are concentrated in direct proportion to the volume of liquid that passes through the membrane. Molecules whose size are close to that of the pores, concentrate to a lesser extent with some of the molecules passing through the membrane in the permeate. The concentration of freely permeable molecules (salts) in the sample remains essentially unchanged.
UV	Ultraviolet Adsorption

Life Sciences

New York — USA
+1 800 717 7255 toll free
+1 516 484 5400 telephone
+1 516 801 9548 fax
biotech@pall.com e-mail

Portsmouth — Europe
+44 (0)23 9230 3303 telephone
+44 (0)23 9230 2506 fax
BioPharmUK@europe.pall.com e-mail

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