

Millipore®

Pellicon® 2 Cassettes

Installation, User, and Maintenance Guide

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Introduction

This guide provides installation and operation procedures for Pellicon® 2 cassettes. Refer to the Certificate of Quality supplied with each cassette for specifications.

Membrane Type and Filtration Area

In multiple-cassette installations, all cassettes must have the same membrane and feed channel screen. Do not mix cassettes with membranes of different pore sizes or nominal molecular weight limits. The area of membrane used depends on the cassette surface area required for the application and is determined during process optimization trials.

Pellicon® 2	Filtration Area (m ²)	
Mini cassette	0.1	
Cassette	0.5	
Maxi cassette	A or C screen	2.5
	V screen	2.0

Nominal Molecular Weight Limit

The retentive abilities of ultrafiltration (UF) membranes are characterized by a nominal molecular weight limit (NMWL). NMWL does not indicate absolute retention/sieving ratings, but can help determine what membrane rating is applicable for a particular process.

- Select a membrane that has a NMWL one-third to one-fifth the molecular weight of the product to be retained.
- If the product must pass through the membrane to the filtrate, select a NMWL three to five times larger than the size of the product.
- A minimum size difference of approximately five times is required between components that are being separated.

Membrane	NMWL (kDa)
Biomax® 5 (PBCC)	5
Biomax® 8 (PBFC)	8
Biomax® 10 (PBGC)	10
Biomax® 30 (PBTK)	30
Biomax® 50 (PBQK)	50
Biomax® 100 (PBHK)	100
Biomax® 300 (PBMK)	300
Biomax® 500 (PBVK)	500
Biomax® 1000 (PBXK)	1000
Ultracel® 5 (PLCCC)	5
Ultracel® 10 (PLCGC)	10
Ultracel® 30 (PLCTK)	30
Ultracel® 100 (PLCHK)	100
Ultracel® 300 (PLCMK)	300

Membrane	NMWL (kDa)
Ultracel® 1000 (PLCXK)	1000
PL regenerated cellulose (PLAC)	1
PL regenerated cellulose (PLBC)	3
PL regenerated cellulose (PLGC)	10

Membrane	Pore Size (µm)
Durapore® (VVPP)	0.1
Durapore® (GVPP)	0.22
Durapore® (HVMP)	0.45
Durapore® (DVPP)	0.65

Effect of NMWL and Membrane Retention on Yield

A protein's stated molecular weight can change based on pH/ionic buffer conditions, protein-to-protein interactions, and protein-contaminant interactions. During process optimization, characterize the feedstock solution to fully understand the potential impact on retention and membrane selection. Product loss to the filtrate due to incomplete retention can be cumulative for the concentration and diafiltration sections of a process. To understand retention changes for batch ultrafiltration and constant diafiltration processes where retention remains constant, use the following calculation:

$$\text{Product Loss in Permeate} = 100 * \{1 - \exp[(R-1)(\ln \text{VCF} + N)]\}$$

Where:

N = Number of diavolumes

VCF = Volumetric concentration factor

R = Product retention

Intercassette Gaskets

Pellicon® 2 mini cassettes (0.1 m²), cassettes (0.5 m²), and maxi cassettes (2.0 or 2.5 m²) are shipped with two 0.044-inch-thick silicone gaskets for use between cassettes. Exceptions are P2C005C25, P2C010C25, and P2C030C25 Pellicon® 2 maxi cassettes (2.5 m²) which are shipped with two 0.088-inch-thick silicone gaskets for use between cassettes.

Water Quality

Reverse osmosis (RO) water or water for injection (WFI) is recommended.

Feed Flow Rate

The feed flow rate (L/min per m²) varies based on module and feed channel turbulence promoter. The higher the feed flow rate, the higher the permeate flux at equal TMP. A higher feed flow rate increases the sweeping action across the membrane, which reduces the concentration gradient toward the membrane surface.

Higher feed flow rates cause the product to experience more passes through the pump in a given amount of time, which can lead to product quality degradation. Also, higher feed flow rates require larger pumps and larger-diameter piping. This increases the system holdup volume and could increase product losses due to unrecoverable holdup.

During optimization trials, choose an appropriate combination of feed flow rate and TMP to maximize permeate flux. This minimizes process time and/or membrane area, allowing for optimal membrane area and pump sizing (minimal holdup due to pump sizing as well as pump passes).

Pump Capacity

Select a pump with adequate capacity.

Cassette	Recommended feed flow rates (L/min/m ²)
A and C Screen	4–7
V Screen	7–10

Do not exceed 10 L/min/m²

Optimal feed flow rate and transmembrane pressure (TMP) depend on the characteristics of the product feed stream being filtered and should be determined during optimization trials.

Transmembrane Pressure

The average applied pressure from the feed to the filtrate side of the membrane.

$$\text{TMP} = [(\text{PF} + \text{PR})/2] - \text{Pf}$$

Where:

TMP = Transmembrane pressure

PF = Feed pressure

PR = Retentate pressure

Pf = Filtrate pressure

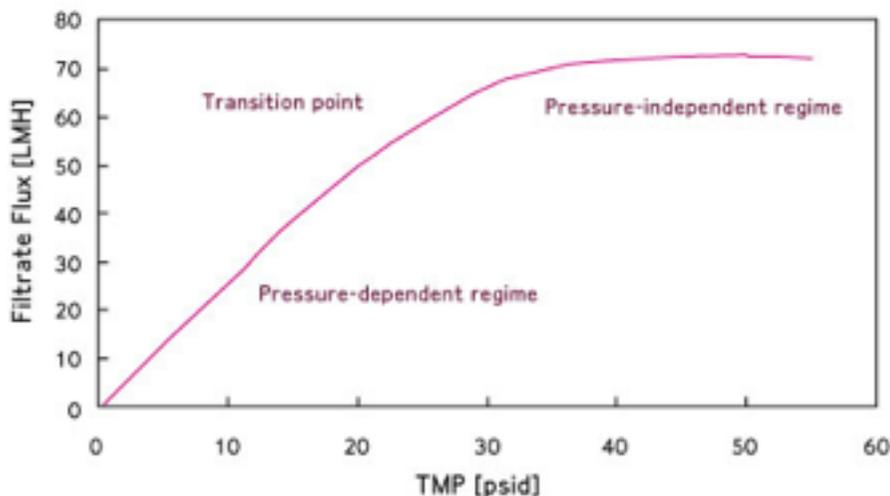
Flux Excursion

The relationship between flux and transmembrane pressure can be understood by generating a flux excursion curve, which is a plot of the permeate flux versus transmembrane pressure at constant feed flow rate:

- The first part of the curve is where the flux increases with increasing pressure (TMP). This is the pressure-dependent regime.
- The level part of the curve (knee of the curve) is the pressure-independent regime. At this point, there is no gain in flux made by increasing pressure.
- When flux starts to plateau at the knee of the curve, the optimum process TMP operating point has been identified.

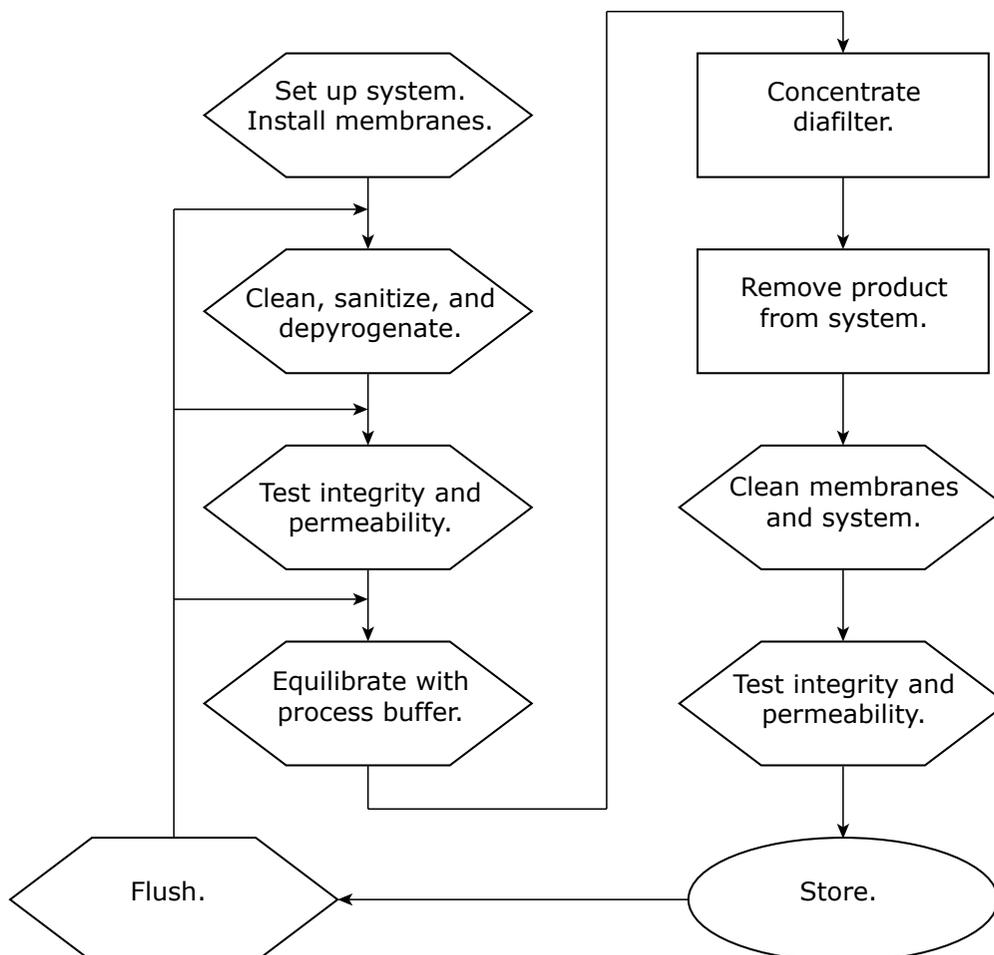
If the process is run with a TMP setpoint in the pressure-independent regime, maximum flux can be achieved, and the required membrane area can be minimized. However, at this point, the protein wall concentration is high and could exceed a solubility limitation, leading to yield losses. Additionally, running under these conditions could cause fouling and eventual decrease in flux. However, if a TMP setpoint in the pressure-dependent regime is used, fluxes are lower and more membrane area is required.

Ideally, for a standard ultrafiltration/diafiltration process, the optimum TMP to run a process is at the knee of the curve. This is the point where nearly the highest flux is achieved without exerting excessive pressure or reaching exceedingly high protein wall concentrations (polarization) or fouling.



Preparing Pellicon® 2 Cassette Filters for Use

This flow chart shows the recommended prep, cleaning and testing steps, which are detailed in this guide, for Pellicon® 2 cassettes.



Handling the Cassettes

The cassettes are fragile. Avoid touching the cassette membrane surface. If a cassette is dropped or the membrane surface impacted, replace the cassette.

Flush and Cleaning

Flush, clean, and fully wet Pellicon® 2 cassettes before processing product to prevent contamination with storage solutions or manufacturing residues (see [Flushing and Cleaning](#)).

Normalized Water Permeability Test

Perform a normalized water permeability (NWP) test to monitor cleaning effectiveness (see [Measurement of Normalized Water Permeability \(NWP\)](#)).

Integrity Testing

Perform an integrity test before and after using Pellicon® 2 cassettes. This test can detect gross device or membrane defects and installation issues (see [Integrity Testing](#)).

Preconditioning

To optimize performance, precondition the membrane by circulating a buffer or medium that closely matches the product to be filtered through the system, including fluid temperature, pH, and osmolarity. Preconditioning the cassettes improves product solubility, prevents product precipitation and absorption during startup, and maximizes product recovery.

Inspecting Tie Rods and Nuts

Inspect the tie rods and bronze nuts for burrs or stripped threads. Nuts should turn freely on the tie rods for proper tightening of the holder.

Replacing Intercassette Gaskets

Two silicone intercassette gaskets are supplied with each cassette, refer to table below for replacement information. For catalog numbers refer to [Accessories and Spare Parts](#).

Pellicon® 2 Cassette Gasket Options

Description	Gasket Thickness mm (in.)	Device
No alignment tabs (wings) for mini holders only	1.1 (0.044)	Pellicon® 2 mini 0.1 m ² cassettes
No alignment tabs (wings) for benchtop holders only	1.1 (0.044)	All Pellicon® 2 0.5 m ² cassettes
With alignment tabs	1.1 (0.044)	All Pellicon® 2 Biomax®, Ultracel® and regenerated cellulose membrane, A and C screen, 2.5 m ² devices except for Ultracel® 5, 10, 30 kD C screen 2.5 m ² devices
Double thick, with alignment tabs	2.2 (0.088)	Ultracel® 5, 10, 30 kD C Screen 2.5 m ² devices: P2C005C25, P2C010C25, P2C030C25

Installing Pellicon® 2 Mini Cassette

Install Pellicon® 2 mini cassettes only in a Pellicon® 2 mini cassette holder.

Equipment

- Pellicon® 2 mini holder, shipped with standard tie rods
- Torque wrench
- Hex-type socket, $\frac{9}{16}$ inch deep, supplied with the Pellicon® 2 mini holder
- Gaskets, supplied with the Pellicon® 2 mini cassette (For replacements, refer to [Replacing Intercassette Gaskets.](#))
- Spacers, supplied with the Pellicon® 2 mini holder

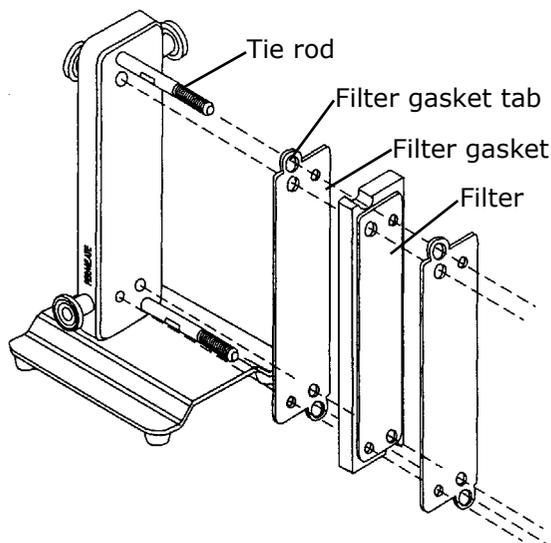
NOTE

The number of spacers needed depends on the number of cassettes installed. Use additional spacers with long tie rods when installing minimum membrane area.

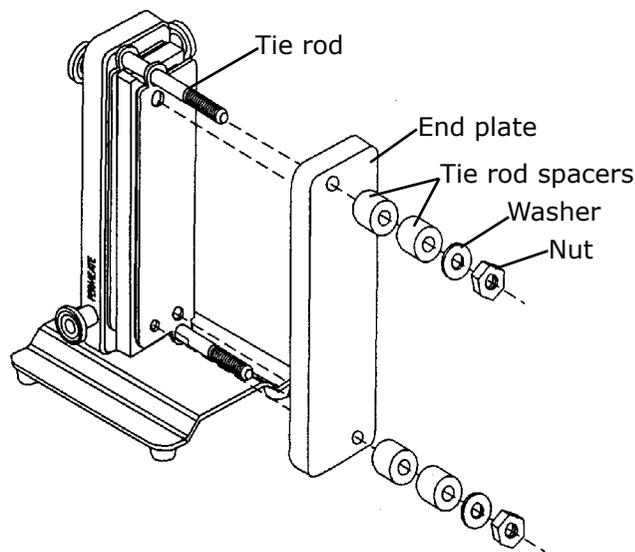
Pellicon® 2 Mini Holder Tie Rod Options

Description	Length (mm)	Number of Pellicon® 2 0.1 m ² Cassettes	
		A and C screen	V screen
Pellicon® 2 mini holder standard tie rods	103	1 to 3	
Pellicon® 2 mini holder long tie rods	141.6	1 to 5	
Cogent® M1 mini holder long tie rods	200	1 to 8	

Installation



Installing the intercassette gasket



Attaching the end plate

1. Remove the clear protective film from both sides of the silicone gasket. Wet the gasket with water if required.
2. Slide the gasket tabs over the tie rods and press the gasket against the base plate, as shown in the illustration.
3. Ensure that the large feed and retentate holes on the silicone gasket align to the large feed and retentate holes on the holder base plate.
4. Carefully remove the cassette from the packaging. Handle the cassette by the jacket sides to prevent damage to the exterior screen and membrane. Align the cassette with the tie rods. Install the cassette so it is parallel with the base plate.

NOTE

The cassette will extend beyond the edges of the holder end plate if the cassette is not properly aligned to the feed, retentate, and permeate holes.

5. Repeat step 1 and slide the silicone gasket tabs over the tie rods. Gently align the gasket against the cassette.
6. Repeat steps 4 and 5 for additional cassettes.
7. Slide the end plate onto the tie rods and press the end plate against the gasket.
8. Place an equal number of spacers on each tie rod. Use tie rod spacers when operating the system with low membrane area. Place enough spacers on each tie rod so the nuts are screwed less than the length of the hex socket on the tie rods to tighten the holder.
9. Place one washer and one nut on each tie rod. Hand tighten the nuts evenly by alternating from one to another.
10. Tighten the holder with the torque wrench set between 20.3 and 22.6 Newton-meters (180 and 200 inch-pounds).

11. Turn each nut $\frac{1}{4}$ turn with the torque wrench, alternating from one nut to the other until the torque wrench clicks when it reaches the set point.
12. Wait 5–10 minutes to allow the compression to stabilize. Retorque to 20.3 and 22.6 Newton-meters (180 and 200 inch-pounds).

Installing the Pellicon® 2 Cassette and Maxi Cassette in a Benchtop Holder

Equipment

- Stainless steel holder, shipped standard with long tie rod or
Acrylic holder, shipped standard with short tie rod
- Torque wrench
- Hex-type socket, $^{15}/_{16}$ inch deep, supplied with the holder
- Stainless steel support plate, 2 pack, one per holder base
- Gaskets, supplied with the Pellicon® 2 cassette (For replacements, refer to [Replacing Intercassette Gaskets.](#))
- Spacers, supplied with the Pellicon® 2 benchtop holder

NOTE

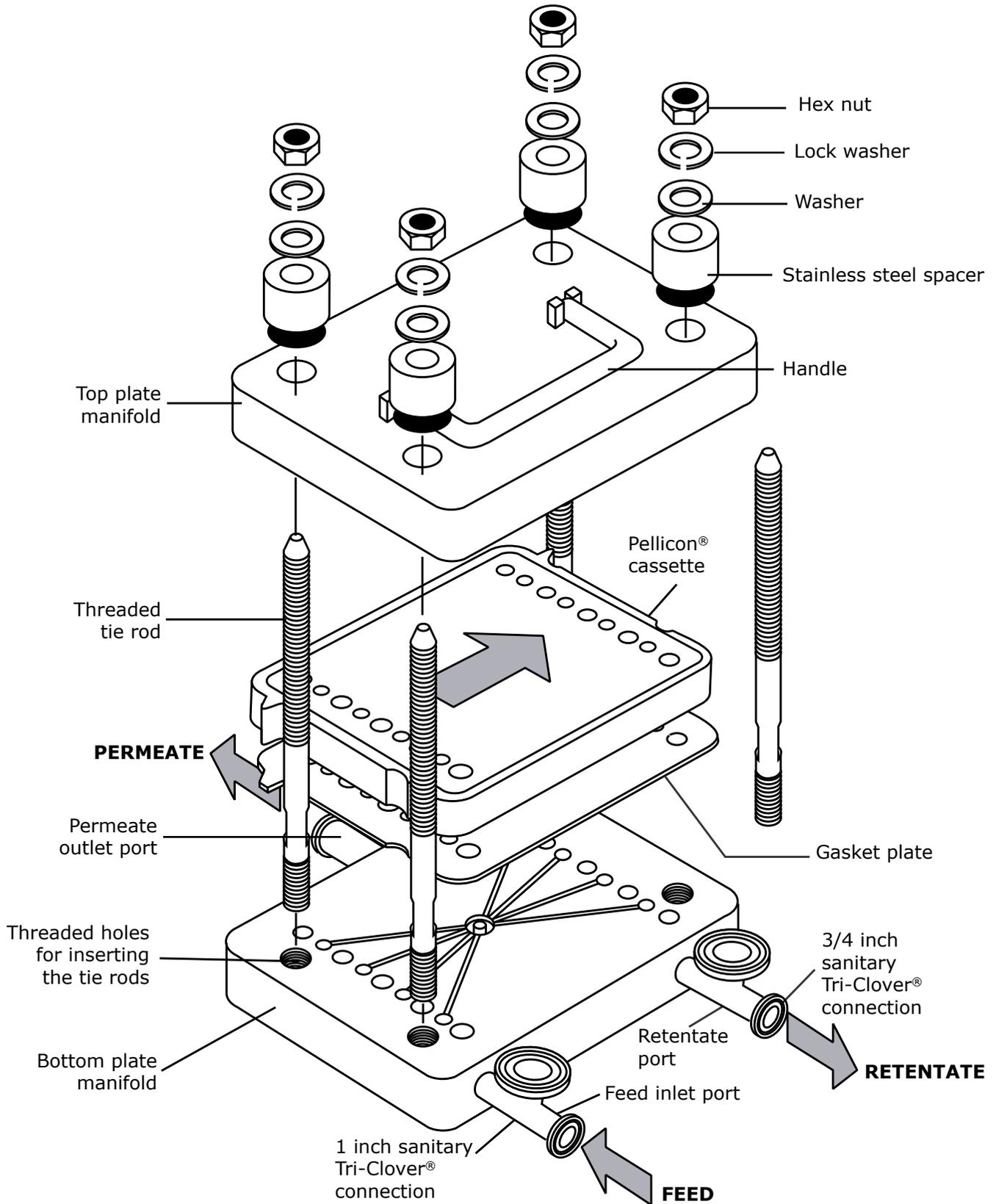
The number of spacers needed depends on the number of cassettes installed. Use additional spacers with long tie rods when installing minimum membrane area.

Pellicon® 2 Cassette and Maxi Cassette Tie Rod Options

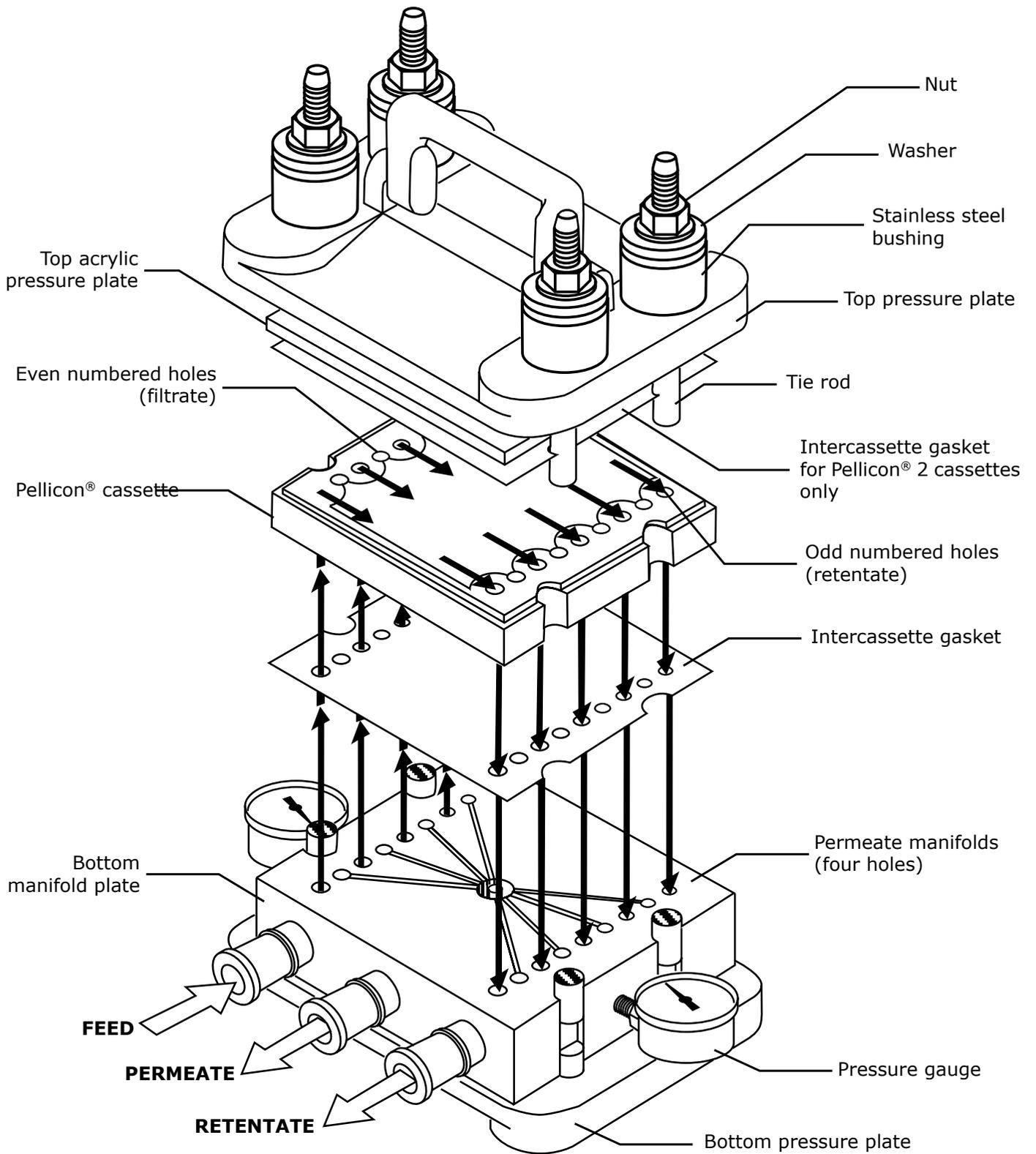
Description	Length (inches)	Number of Pellicon® 2 Cassettes	
		A and C screen	V screen
Long tie rods for stainless steel holder	10.50	5 to 10 x 0.5 m ² 1 to 2 x 2.5 m ²	5 to 10 x 0.5 m ² 1 to 2 x 2.0 m ²
Short tie rods for stainless steel holder	7.00	1 to 5 x 0.5 m ² 1 x 2.5 m ²	1 to 5 x 0.5 m ² 1 x 2.0 m ²
Long tie rods for acrylic holder	12.50	5 to 10 x 0.5 m ² 1 to 2 x 2.5 m ²	5 to 10 x 0.5 m ² 1 to 2 x 2.0 m ²
Short tie rods for acrylic holder	8.75	1 to 5 x 0.5 m ² 1 x 2.5 m ²	1 to 5 x 0.5 m ² 1 x 2.0 m ²

Installation

Stainless steel Pellicon® 2 Cassette holder



Acrylic Pellicon® 2 Cassette holder



1. For acrylic holder installation only, place the bottom acrylic manifold onto the aluminum base. The manifold is part of the base in the stainless steel holder.
2. Remove the protective clear polyethylene film from both sides of the silicone gasket. Place the silicone gasket on the bottom manifold plate. Optional: Use highly purified water to wet the gasket.
3. Install one stainless steel support plate onto the holder base. This plate will ensure a good seal between the holder and cassette ports.
4. Remove the protective clear polyethylene film from both sides of the silicone gasket. Place the silicone gasket on top of the stainless steel plate. Optional: Use highly purified water to wet the gasket.
5. Carefully cut the vacuum sealed bag and remove the cassette. Handle the cassette by the jacket sides to prevent accidental damage on the exterior screen and membrane. Place the cassette on top of the silicone gasket. The notches on the sides of the cassette align with the tie rods on the holder.

NOTE

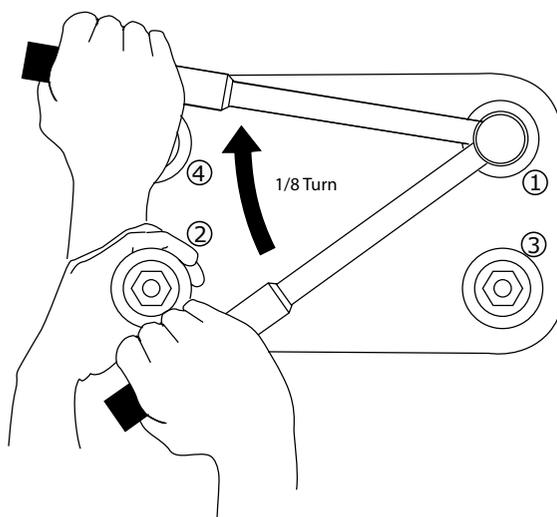
Orient the cassette so that the label is at the front of the holder, right side up, for easier reading.

6. When using more than one cassette in the holder, repeat steps 4 and 5. End with a silicone gasket on top of the final cassette.
7. For acrylic holders only, install the top acrylic plate on top of the last gasket.
8. Place the top aluminum pressure plate (acrylic holders) or the top stainless steel plate (stainless steel holders) with the slotted or handle side facing up.
9. Place onto the tie rods, in this sequence, the tie rod spacers (as needed), washers, and bronze hex nuts.

NOTE

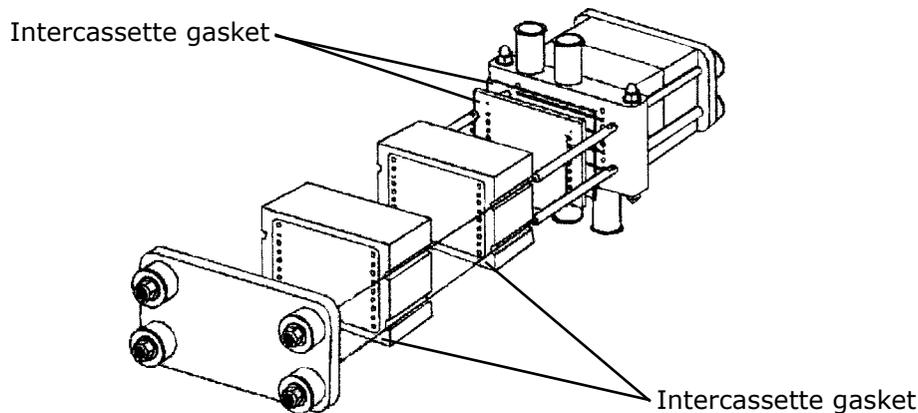
Tie rod spacers are required when operating the system with low membrane area. Place enough spacers evenly on each tie rod so that, when the holder is tightened, the nuts are screwed less than the length of the hex socket on the tie rods. The number of spacers needed depends on the number of cassettes installed.

10. Hand tighten the nuts in a diagonal pattern as evenly as possible, in the sequence shown in the illustration.



11. Set the torque wrench to 40 to 45 Nm (350–400 in-lb).
12. Initially turn each nut to a $\frac{1}{2}$ or $\frac{3}{4}$ turn, in the sequence shown in the illustration.
13. As it becomes more difficult to tighten each nut, use $\frac{1}{8}$ to $\frac{1}{4}$ turns in the same sequence.
14. After each nut reaches set point of 40 to 45 Nm (350–400 in-lb), the wrench will click.
15. Wait 5–10 minutes to allow system compression to stabilize. Retorque to 40 to 45 Nm (350–400 in-lb).
16. The system can be retorqued up to a maximum of 62 Nm (550 in-lb) as needed to ensure a liquid tight seal or if the installation fails its integrity test.

Installing the Process Scale Holder



Equipment

- Manual or hydraulic enclosure holder
- Torque wrench
- Socket (manual holder only)
- Stainless steel support plate
- Gaskets, supplied with the Pellicon® 2 maxi cassette (For replacements, refer to [Replacing Intercassette Gaskets.](#))

Up to four Pellicon® 2 maxi cassettes (2.5 m² or 2.0 m² for V screen) can be installed on each side of the holder (eight maxi cassettes per level).

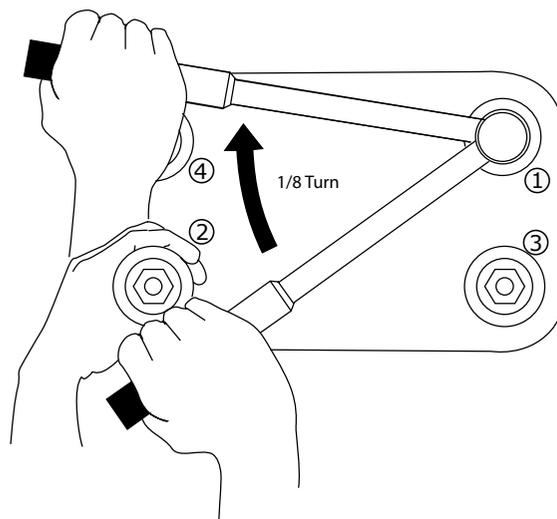
Installation

1. Loosen the nuts on the tie rods of the holder and remove the nuts, washers, and end plate.
2. Remove the protective clear polyethylene film from both sides of a silicone gasket. Optional: Wet the gasket with highly purified water. Install the gasket on one side of the center manifold block by slipping the wings over the tie rods (manual holder) or alignment rods (hydraulic holder). Ensure the gasket is flat and align the holes in the gasket with those on the manifold.
3. Install one stainless steel support plate on top of the gasket.
4. Carefully remove the protective clear polyethylene film from both sides of a silicone gasket. Optional: Wet the gasket with purified water. Install the gasket on top of the stainless steel support plate by slipping the wings over the tie rods (manual holder) or alignment rods (hydraulic holder). Ensure the gasket is flat. Align the holes in the gasket with the holes on the manifold.

5. Remove the Pellicon® 2 cassette from the vacuum sealed bag. Handle the cassette by the jacket sides to prevent accidental damage on the exterior screen and internal membrane. Place the cassette against the silicone gasket. The notches on the sides of the cassette align with the tie rods on the holder. When using more than one cassette in the holder, place one silicone gasket between every single cassette, ending with a silicone gasket against the final cassette.
6. Slide the stainless steel end plate against the final gasket.
7. Place tie rod spacers as needed. Place a washer and a nut onto each tie rod.
8. Hand tighten the nuts in a diagonal fashion as evenly as possible, in the same sequence as for torquing (see step 10 in Installing the Pellicon® 2 cassette and maxi cassette in a Benchtop Holder, [Installation](#)).

Tightening a Manual Holder

1. Set the torque wrench to 40 to 45 Nm (350–400 in-lb).
2. Turn each nut $\frac{1}{2}$ or $\frac{3}{4}$ turn in the sequence shown in the illustration.



3. As it becomes harder to tighten each nut, use $\frac{1}{8}$ to $\frac{1}{4}$ turns, in the same sequence.
4. After each nut reaches set point of 40 to 45 Nm (350–400 in-lb), the wrench will click.
5. Wait five to ten minutes to allow system compression to stabilize. Retorque to 40 to 45 Nm (350–400 in-lb).
6. The system can be retorqued up to a maximum of 62 Nm (550 in-lb) as needed to ensure a liquid-tight seal or if the installation fails the integrity test.

Tightening a Hydraulic Process Scale Holder

The hydraulic closure with manual controls is intended for use with the Pellicon® 2 process scale cassette holder. It maintains appropriate pressure on the holder during processing or during storage of installed cassettes.

Use **run** mode for process operations. The appropriate hydraulic compression for process and cleaning operations is 134 + 6.7 bar (1950 ± 100 psig).

1. Install the grooved tie rods, Triclover clamps, clamp inserts, and the hand tightening wheels.

NOTE

Only hand tighten the hand wheels. Do not use a wrench. Ensure that each end plate is flush against the cassette assembly and both hand wheels are snug against the end plate. At least one inch of excess thread on the clamp rod exiting the hand wheel should be exposed.

2. Repeat the procedure for any other holder levels.
3. Set the system to **run** mode to compress the cassettes.

NOTE:

The hydraulic compression range of 134 + 6.7 bar (1950 ± 100 psig) is specific to Pellicon® 2 installation with our hydraulic piston design. A different set point will be needed if installing Pellicon® 2 cassettes into other holders.

Pellicon® 2 Holder Assembly

Maintenance

Pellicon® 2 cassettes must be operated while under compression from the holder assembly to ensure that the cassettes seal properly. Compression is supplied by applying torque to the nuts on the threaded tie rods of the holder assembly.

Proper holder maintenance will prevent the necessity for higher torque values to achieve proper sealing.

1. Clean nuts, washers, and tie rods before every use to ensure that they are free of particles. Use a mild solvent such as IPA and water.
2. Apply a few drops of suitable lubricant on the tie rods and between the nuts and the washers whenever permitted at specified intervals per standard operating procedures. Use food-grade vegetable oil or glycerin.

NOTE

Check quality assurance protocols before introducing a new lubricant.

3. Protect the tie rods from receiving any blows that could damage their threads, which can lead to galling. Do not damage tie rods when using a torque wrench.
4. Replace any component that is visibly worn or does not spin freely.
5. Replace nuts and washers after every ten installations to maintain high sealing force in the holder.
6. When new nuts do not spin freely, refurbish the threaded rods with a $\frac{5}{8}$ in.-18 die.
7. Keep a set of spare on hand at all times.

Flushing and Cleaning

These flushing and cleaning procedures are recommended guidelines. Evaluate flushing and effective cleaning procedures for each process.

Flush and clean all cassettes before first use.

Flush Pellicon® 2 cassettes before and after filtration processes and cleaning to remove the chemical agents in that step. When multiple solutions are required for cleaning and sanitization, flush each cleaning agent completely from the system before introducing the next cleaning agent.

Use a cleaning agent that is compatible with the membrane and device.

1. Fill the recycle tank with highly purified water, water for injection (WFI), or reverse osmosis (RO) water. Extremely pure flush water prevents fouling the membranes and the introduction of contaminants into the system.
2. Open the retentate valve completely and direct the retentate line to drain.
3. Direct the permeate line to drain.
4. Turn on the feed pump and pump water into the feed port of the Pellicon® 2 holder. Recommended feed flows for flushing, cleaning, storing, and all process steps are detailed in the following table. Determine actual process flow rates during optimization trials.

Feed Screen	Feed Flow (L/min/m ²)
A	4.0-6.0
C	4.0-7.0
V	7.0-10.0

NOTE These flow rates are appropriate for most typical applications; however, some processes may require flow rates outside of these ranges. Do not exceed 10 L/min/m².

5. Membrane flushing and cleaning is detailed in the following table:

Operation	Step	Volume per m ² (L)	Solution
Initial preservative or storage solution flush	Single pass with permeate open*	≥20	WFI, RO, or HPW
Cleaning and/or sanitization	Single pass with permeate open*	10	See Recommended Cleaning Agents and Conditions for Pellicon® 2
	Total recirculation** (30-60 min)	5 to 10	
Post cleaning and/or sanitization Flush	Single pass with permeate open*	10 to 20	WFI, RO, or HPW
Final flush and NWP measurement	Single pass with permeate open*	≥10	
Flush until user conductivity specification is met (if applicable)	Total recirculation** (10-15 min) with NWP measurements	10	

***Single pass with permeate open:** Permeate and retentate valves are open. Flows are directed to drain. Adjust

the retentate valve so that 30–50% of the feed flow is converted to filtrate. This ratio of filtrate to feed is defined as the conversion ratio.

****Total recirculation:** Permeate and retentate flow paths are directed to the recycle tank. After first 12 L/m² have flowed through retentate side, partially close the retentate valve to achieve 30–50% conversion.

Additional flush volumes, recirculation, and hold steps can be performed to ensure that specific flush requirements are met.

If the system is complex and has other associated manifolds, ensure that all wetted surfaces in the manifolds are exposed to the solution. All valves exposed to process fluids should also be exposed to the cleaning and flushing solutions. Cycle (partially open and then partially close) valves at least twice over the course of the cycle to ensure that all wetted internal surfaces of the valve body are exposed to the solutions.

Take samples from the retentate and permeate after the final flush to determine the level of storage solution and/or cleaning solution removed from the cassette.

For proper cleaning of cassettes, sodium hydroxide is the most common cleaning agent. It is effective for removing proteins and nucleic acids and for inactivating most viruses, bacteria, yeast, fungi, and endotoxins. It can be easily detected, removed, and disposed of.

Recommended Cleaning Agents and Conditions for Pellicon® 2

Membrane	Cleaning Agent	Concentration	pH	Temperature (°C)	Time (minutes)
PLAC, PLBC*	NaOH	0.05 N	12.7	Ambient	15–30
PLGC	NaOH	0.1 N	13	20–50	30
Ultracel®	NaOH	0.1 N	13	20–50	30
Biomax®	NaOH	0.1–1.0 N	13–14	20–50	30–60
Ultracel®, Biomax®, Durapore®	Peracetic Acid	100–200 ppm	3.5	20–50	15–30
Biomax®, Durapore®	NaOCl	50–250 ppm (active chlorine)	7–8	20–50	15–30

*Milder cleaning conditions can extend the life of regenerated cellulose membranes with a nominal molecular weight limit of 1, 3, or 5 kDa. Use ambient temperatures and the lowest NaOH concentration possible. Better membrane life has been observed at 20–25 °C for PLAC and PLBC membranes and 25–40°C for PLGC, etc.

Alternate Cleaning Conditions and Agents for Pellicon® 2 Filters

Membrane	Cleaning Agent	Concentration	pH	Temperature	Time (min)
Biomax®	NaOH/NaOCl	0.1N/250 ppm to 0.5N/600 ppm	13	20–50 °C	30
Durapore®	NaOCl – This step must be followed by a water flush and an acid recirculation.	100–500 ppm followed by 0.1N phosphoric acid	10–11	20–50 °C	30

Cleaning Agents Based on Application

Process Fluid	Probable Foulants	Cleaning Agent	
		Recommended	Alternate
Pellicon® 2 with ultrafiltration membranes			
Protein solutions Blood and serum products, bacterial and mammalian products, enzymes, vaccines, viruses	Adsorbed protein	NaOH NaOCl*	N/A
Bacterial cell whole broths <i>E Coli, bacillus, other</i>	Adsorbed protein, antifoams, cell debris, lipids, polysaccharides	NaOH followed by NaOCl*	
Bacterial lysates	Protein, lipopolysaccharides, cell debris	NaOH followed by H ₃ PO ₄	
		NaOCl* followed by H ₂ PO ₄	
Nucleic acids	N/A	H ₃ PO ₄	
Polysaccharides lipopolysaccharides, dextrans, pectins, starches, mucopolysaccharides	Adsorbed polysaccharide	NaOH, NaOCl* Tergazyme®	
Depyrogenation radio opaque imaging agents, antibiotics, low-molecular-weight solutions	Residual organic colloids, lipopolysaccharides	NaOH	
Food and beverage streams Food, wine, proteins, vinegar, juice	Protein, tannins, phenolics, organic colloids, humic acids	NaOH followed by NaOCl*	
		Tergazyme® followed by NaOCl*	
Water treatment	Iron complexes	Citric acid HNO ₃	
	Mineral scale, inorganic deposits	HNO ₃ citric acid H ₃ PO ₄	
Pellicon® 2 Cassette Filters with microporous Durapore® membranes			
Protein clarification Recombinant proteins, blood and serum products, pre-column clarification	Precipitated protein, lipoproteins and lipids	NaOCl, Tween® 80	Triton® X-100, Tergazyme, Urea
Mammalian cell culture	Cell debris	NaOCl	H ₃ PO ₄
Red blood cell ghosts	Cell wall debris, precipitated protein	NaOCl, Tergazyme®	N/A

Process Fluid	Probable Fouling	Cleaning Agent	
		Recommended	Alternate
Bacterial cell broths mycelial, fungal fermentation broths	Absorbed protein, antifoams, cell debris, lipids, polysaccharides, media ingredients	NaOCl, Tergazyme® followed by NaOCl	Triton® X-100 or SDS followed by NaOCl
Bacterial lysate clarification	Protein, lipopolysaccharides, cell debris	NaOCl followed by H ₃ PO ₄	Tween® 80, Triton® X-100 or SDS followed by NaOCl
Pre-chromatographic clarification	Insoluble denatured protein	NaOCl Tween® 80 followed by NaOCl	Triton® X-100
Polysaccharides Lipopolysaccharides, Dextrins, Pectins, Starches, Mucopolysaccharides	Adsorbed polysaccharide	NaOCl or Tergazyme®	Triton® X-100 or SDS followed by NaOCl
Juice and beverage clarification	Pectin, starches, polyphenolics, protein complexes, colloids	NaOCl*	N/A
Water treatment	Iron complexes, mineral scale	Citric acid	HNO ₃
Clarification of inorganic precipitates	Mineral scale inorganic deposits	HNO ₃ , H ₃ PO ₄	Citric acid

*Not for use with any cellulose membranes.

Cleaning Conditions

Fouling	Membrane	Cleaning Agents	Concentration	Temperature °C	pH	Time (minutes)
Organics, biofilms, biopolymer, proteins polyphenolic	Biomax®	NaOH	0.1–0.5N	40–50	13–13.7	30–60
	PLAC, PLBC		0.1N	20–25	13	
	Ultracel®					
Proteins, biopolymers polysaccharides	Biomax® Durapore®	NaOCl	250 ppm active chlorine	40–50	10–11	30–60
Biopolymers proteins, colloidal deposits, polyphenolic fats, oils, grease, antifoams, scale	All	Tergazyme®	0.2%	40–50	9–10	30–60
Proteins, lipids, lipopolysaccharides, oils, antifoams	All	Triton®-X 100	0.1%	40–50	5–8	30–60
		SDS				
		Tween® 80				
Proteins, protein precipitates	All	Urea	7M	40–50	8	60

Foulants	Membrane	Cleaning Agents	Concentration	Temperature °C	pH	Time (minutes)
Mineral scale, nucleic acids	All	HNO ₃	0.1N	40-50	1.0	30
Iron, manganese, scale	All	Citric acid (adjust to pH 3 with NH ₄ OH)	1%	40-50	3.0	60

Sanitization

Always sanitize after the system has been thoroughly cleaned and flushed before processing.

Sanitization pressures, flow rates, and volumes are identical to those used for cleaning (see [Flushing and Cleaning](#)).

Select a sanitization agent based on membrane compatibility.

Membrane Type	Sanitization Agent	Concentration	pH	Temperature °C
Biomax®	NaOCl	100–500 ppm	10–11	20–50
Durapore®	NaOCl NOTE: This step must be followed by a water flush and an acid recirculation.	100–500 ppm	10–11	20–50
All Membranes	Peracetic acid	50–200 ppm	—	20–25
Biomax®	NaOH	0.5–1.0 N	13.7–14	20–50
Ultracel®		0.1 N	13	

NOTES

Use sanitization agents and conditions that are suitable for the process requirements. Sanitization agents must comply with applicable local regulations.

Milder cleaning conditions can extend the life of regenerated cellulose membranes with a nominal molecular weight limit of 1, 3, or 5 kDa. Use ambient temperatures and the lowest NaOH concentration possible.

Depyrogenation

If depyrogenation is required, perform only after the system has been cleaned, sanitized, and flushed. Depyrogenation pressures, flow rates, and volumes are identical to those used during cleaning (see [Flushing and Cleaning](#)). Use water for injection or reverse osmosis water. Based on membrane compatibility, select a depyrogenation agent from the table below.

Depyrogenation Conditions and Agents

Membrane	Sanitization Agent	Concentration	Temperature °C	pH	Time (minutes)
Biomax®	NaOH	0.1-1.0N	30-50	13-13.7	30
Ultracel®	NaOH	0.1N	30-40	13	30
Biomax®, Durapore®	NaOCl	300 ppm active chlorine (600 ppm NaOCl)	30-50	10-11	30
All	H ₃ PO ₄	0.1N	30-40	1.0	30

Measurement of Normalized Water Permeability (NWP)

Normalized water permeability (NWP) is the measurement of clean water through a membrane at certain pressure and temperature conditions. Determine the reference measured NWP for Pellicon® 2 membranes prior to the first use of each cassette after exposure to the cleaning solution. Clean and flush new membranes before measuring NWP. Initial NWP is used as a benchmark against which subsequent water permeability measurements can be compared.

A permeability measurement can be taken in a different solution such as storage solution or equilibration buffer (this is typically more convenient at large scale). Consistently take permeability measurements in the same solution and under the same feed flow and pressure conditions so that each subsequent value can be compared to the initial measurement.

1. Take the NWP measurement during the last total recirculation step of membrane flushing and cleaning.
2. Fill the tank with clean water, preferably at 25 °C, or the storage solution or buffer. Take the permeability measurement in the same solution and under the same process conditions every time.
3. Set the feed pump to the feed flow rate used during processing.
4. Partially close the retentate valve to achieve the retentate pressure listed. Higher pressures are necessary for lower molecular weight limit membranes. For more open membranes > 50 kDa adjust the retentate value to generate 5 psi retentate pressure and then control the permeate flow to the target 100–150 L/hr/m² (LMH) or approximately 30% conversion of the feed flow into permeate flow.

Membrane NMWL	Retentate Pressure (psi)
1, 3, 5 kDa	20–30
10 kDa	5–15
30 kDa	1–5
> 50 kDa (open UF and MF membranes)	5, then use permeate control to restrict filtrate flux to 100–150 LMH or 30% conversion of feed to permeate flow

5. Recirculate the fluid for 5–10 minutes. Ensure that the flow, pressure, and temperature conditions are stable.
6. Record the feed and permeate flow rates, feed, retentate and permeate pressures, and the temperature of the fluid.
7. Use the following equation to calculate the NWP:

$$NWP = \frac{R \cdot F}{A \cdot \left\{ \left[\frac{P_{in} + P_{out}}{2} \right] - P_p \right\}}$$

This yields LMH/psi [liters/m²/hours/psi]

Where:

R = Permeate flow rate in L/hour

P_{in} = Feed pressure in psi

P_{out} = Retentate pressure in psi

P_p = Permeate pressure
(if non-zero) in psi

A = Total filter area in m²

F = Temperature correction factor from the NWP temperature correction factor (F)* table.

8. After the first use of the cassette, the NWP should be 60–120% of the original NWP. The NWP should then stabilize for future runs or slowly decrease in a reproducible, stable fashion. It is recommended that the cassette be discarded before 50% of the reference NWP is reached.

NOTE

Cleaning effectiveness, cleaning optimization, and the fouling potential of the product stream all impact on how rapidly the NWP falls upon cassette reuse. Unstable or irreproducible NWP measurements between membrane sets is a sign that the cleaning procedure is not effective.

NWP Temperature Correction Factor (F)*								
T (°F)	T (°C)	F	T (°F)	T (°C)	F	T (°F)	T (°C)	F
125.6	52	0.595	96.8	36	0.793	68.0	20	1.125
123.8	51	0.605	95.0	35	0.808	66.2	19	1.152
122.0	50	0.615	93.2	34	0.825	64.4	18	1.181
120.2	49	0.625	91.4	33	0.842	62.6	17	1.212
118.4	48	0.636	89.6	32	0.859	60.8	16	1.243
116.6	47	0.647	87.8	31	0.877	59.0	15	1.276
114.8	46	0.658	86.0	30	0.896	57.2	14	1.310
113.0	45	0.670	84.2	29	0.915	55.4	13	1.346
111.2	44	0.682	82.4	28	0.935	53.6	12	1.383
109.4	43	0.694	80.6	27	0.956	51.8	11	1.422
107.6	42	0.707	78.8	26	0.978	50.0	10	1.463
105.8	41	0.720	77.0	25	1.000	48.2	9	1.506
104.0	40	0.734	75.2	24	1.023	46.4	8	1.551
102.2	39	0.748	73.4	23	1.047	44.6	7	1.598
100.4	38	0.762	71.6	22	1.072	42.8	6	1.648
98.6	37	0.777	69.8	21	1.098	41.0	5	1.699

*Based on water fluidity relative to 25 °C (77 °F) fluidity value $F = (\mu_{T_{°C}}/\mu_{25_{°C}})$ or $(\mu_{T_{°F}}/\mu_{77_{°F}})$.

Average Water Permeability at Room Temperature for Pellicon® 2 Cassette Filters

These water permeability values are based on a membrane coupon tested under controlled conditions. System and device variables can modify the water permeability values greatly; these values are for guidance only.

Membrane and NMWL	Average Water Permeability	
	(LMH/psi)	(LMH/Bar)
Biomax® 5	5.0	73
Biomax® 8	11.5	167
Biomax® 10	20.0	290
Biomax® 30	24.0	348
Biomax® 50	28.0	406
Biomax® 100	39.0	566
Biomax® 300	50.0	725
Biomax® 500	56.0	819
Biomax® 100	63.0	914
PL Regenerated Cellulose (PLAC)	0.3	4.4
PL Regenerated Cellulose (PLBC)	0.6	8.7
PL Regenerated Cellulose (PLGC)	4.0	58
Ultracel® 5	0.9	13.1
Ultracel® 10	6.4	93
Ultracel® 30	10.5	152
Ultracel® 100	30.0	435
Ultracel® 300	50.0	725
Ultracel® 1,000	60.0	870

Integrity Testing

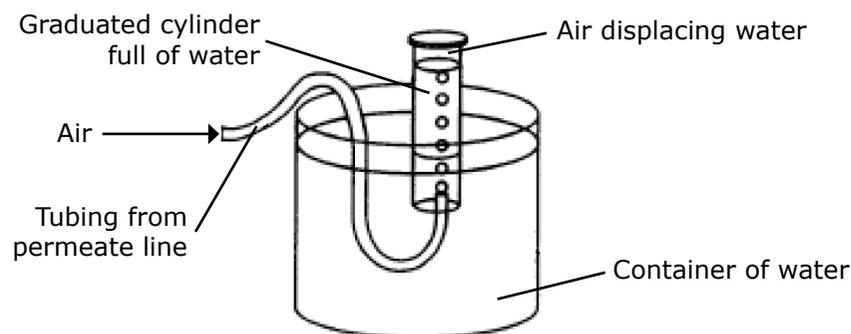
Test cassette integrity on a cleaned and thoroughly flushed system. The presence of residual cleaning agents and/or process residues can significantly alter integrity test results. Integrity test pressure and air diffusion specifications for each membrane can be found on the Certificate of Quality supplied with the cassette or in the table in this section.

1. Ensure that the system is thoroughly cleaned and that the membrane is thoroughly wetted. Ensure thorough wetting by recirculating water for 5–10 minutes at the conditions specified in [Measurement of Normalized Water Permeability \(NWP\)](#).
2. Drain the system. Drain the feed/retentate side of the system as thoroughly as possible. If possible, manually drain the permeate line. Do not blow down.
3. Attach a regulated and filtered air supply to the feed or retentate side of the holder, preferably to the more elevated end.
4. Isolate either the feed or the retentate manifold (the port not connected to the air supply) by closing a valve or capping the manifold if there is no valve on it. The permeate line should be open at all times.
5. Slowly raise the air pressure to the specified test value and wait 5 minutes or as long as needed to purge residual water in the permeate line and stabilize air pressure.

NOTE

Do not exceed the specified test air pressure. This could displace water from the wetted pores. This would result in excessively high air flow. Rewet the membrane if this occurs.

6. Measure and record the air pressure, ambient temperature, and the air flow rate exiting the permeate line. Measure the air flow rate with an air flowmeter or by measuring the air displaced into a submerged and inverted volumetric cylinder as shown in the following illustration.



7. Compare the measured air flow rate to the specified flow value in the following table. If the measured air flow rate exceeds the specified flow value, confirm that the Pellicon® 2 cassette has been installed and torqued correctly. Rewet and retest. If problem persists, refer to [Troubleshooting](#).

The following table lists air flow rates through a fully water wetted membrane tested with air at ambient temperature.

NOTE

If different test temperature or test gas is used, the air integrity specification must be converted to the conditions. For example, if nitrogen is used as the test gas, the diffusional flow will be 80% of the air test gas values provided below.

Catalog Number	Membrane NMWL (kDa) or Pore Size (µm)	Air Flow Rates (cc/min)				Test Pressure psi (bar)
		0.1 m ² (1 ft ²)	0.5 m ² (5 ft ²)	2.0 m ² (20 ft ²)	2.5 m ² (25 ft ²)	
Biomax® membranes						
P2B005---	5	≤ 4	≤ 18	≤ 72	≤ 90	10 (0.69)
P2B008---	8	≤ 4	≤ 18	≤ 72	≤ 90	10 (0.69)
P2B010---	10	≤ 4	≤ 18	≤ 72	≤ 90	30 (2.1)
P2B030---	30	≤ 4	≤ 18	≤ 72	≤ 90	10 (0.69)
P2B050---	50	≤ 4	≤ 18	≤ 72	≤ 90	10 (0.69)
P2B100---	100	≤ 4	≤ 18	≤ 72	≤ 90	10 (0.69)
P2B300---	300	≤ 12	≤ 60	≤ 240	≤ 300	10 (0.69)
P2B500---	500	≤ 12	≤ 60	≤ 240	≤ 300	10 (0.69)
P2B01M---	1,000	≤ 12	≤ 60	≤ 240	≤ 300	10 (0.69)
Regenerated cellulose membrane						
P2PLAC---	1 (PLAC)	≤ 10	≤ 48	≤ 192	≤ 240	30 (2.1)
P2PLBC---	3 (PLBC)	≤ 10	≤ 48	≤ 192	≤ 240	30 (2.1)
P2PLGC---	10 (PLGC)	≤ 6	≤ 30	n/a	≤ 150	5 (0.34)
Ultracel® membrane						
P2C005---	5	≤ 7	≤ 35	≤ 145	≤ 175	30 (2.1)
P2C010---	10	≤ 7	≤ 35	≤ 145	≤ 175	30 (2.1)
P2C030---	30	≤ 4	≤ 18	≤ 72	≤ 90	10 (0.69)
P2C100---	100	≤ 4	≤ 18	≤ 72	≤ 90	10 (0.69)
P2C300---	300	≤ 4	≤ 18	≤ 72	≤ 90	10 (0.69)
P2C01M---	1,000	≤ 4	≤ 18	≤ 72	≤ 90	10 (0.69)
Durapore® membranes						
P2VVPP---	0.1 µm	≤ 3	≤ 12	≤ 48	≤ 60	10 (0.69)
P2GVPP---	0.22 µm	≤ 3	≤ 12	≤ 48	≤ 60	10 (0.69)
P2HVMP---	0.45 µm	≤ 3	≤ 12	≤ 48	≤ 60	10 (0.69)
P2DVPP---	0.65 µm	≤ 3	≤ 12	≤ 48	n/a	10 (0.69)

Storing Filters and Systems

Storing the Filters in the System

After the system has been thoroughly cleaned, flushed, and tested, it can be stored until needed for further processing.

- Do not store an uncrated system outdoors or lean or stack the system.
 - Protect the fragile parts of the system, such as the instruments and electrical enclosure front panel, with bubble-pack or cardboard.
 - Protect open pipe inlets and outlets with plastic or stainless steel caps.
 - Storage pressures, flow rates and volumes are identical to those used for cleaning (see [Flushing and Cleaning](#)).
1. Shut off the recirculation pump and clamp the lines to or from the holder or shut the feed, retentate and permeate valves to keep the holder and cassettes full of storage solution.
 2. Store the holder and cassettes at either ambient temperature or 2–8° C.

To store cassettes on the skid in the holder, shut off the recirculation pump and clamp the lines to/from the holder or close the feed, retentate, and permeate valves to keep the holder and cassettes full of storage solution. It is recommended that storage is performed at 2-8 °C, but if this is impractical on the skid, ambient temperature is acceptable if correctly validated.

Storing the Filters without the System

Filters can be removed from the holder for storage. Wear appropriate protective equipment (rubber gloves, safety glasses) when handling the storage solution.

1. Ensure that the cassettes are fully wetted with storage solution.
2. Shut off the recirculation pump and drain the system.
3. Untorque the holder and remove the cassettes.
4. Place the cassettes in a liquid tight container and fill the container with excess storage solution to fully immerse the cassette.
5. Seal the container and store at 2 to 8°C. Do not freeze.

Suitable storage containers include plastic freezer bags with zipper seals, molded polyethylene or polypropylene freezer containers, or plastic pails with locking and sealing lids.

Recommended Storage Solutions and Conditions for Pellicon® 2 Filters

Storage Solution	Membrane Type	Concentration	pH	Time Period Recommended
NaOH	Biomax®	0.1N	13	6 months
	PLAC, PLBC, and PLCCC	0.05N	12.7	6 months
	PLGC	0.1N	13	4 months
		0.05N	12.7	8 months
	Ultracel®	0.1N	13	6 months
		0.05N	12.7	1 year
Benzalkonium chloride	All	0.1%	7	1 year
H ₃ PO ₄	All	0.1N	2	6 months

NOTES

Use sanitization agents and conditions that are suitable for the process requirements. Sanitization agents must comply with applicable local regulations.

Milder cleaning conditions can extend the life of regenerated cellulose membranes with a nominal molecular weight limit of 1, 3, or 5 kDa. Use ambient temperatures and the lowest NaOH concentration possible.

Troubleshooting

Problem	Possible Causes	Suggestions
Integrity test failure	Areas of membrane are incompletely wetted.	Reflush with water and retest (see Measurement of Normalized Water Permeability (NWP) and Integrity Testing).
	Filter compression is inadequate.	
	Installation is improper.	Ensure that intercassette gaskets and adapter plates or permeate plate are installed correctly. Ensure threads, nuts, and tie rods are undamaged and installed correctly as described in the holder user guide.
	Temperature has changed since last torque of holder.	Torque unit again and retest.
	Gasket has become deformed.	Replace the gasket.
	A procedural error has occurred.	Rerun the test. Follow the procedure in Integrity Testing . Ensure that system is fully drained and air pressure has stabilized prior to taking measurements.
	The membrane or cassette is damaged.	Replace any damaged cassette. Replace any cassette that has been dropped.
External liquid leak from installed devices	Filter compression is inadequate (torque or hydraulic pressure), causing an installation error.	<p>If the holder is ours, ensure torque and hydraulic set point are correct.</p> <p>If the holder is from a third-party supplier, contact Technical Service for guidance on the appropriate torque settings.</p>

Problem	Possible Causes	Suggestions
Yield loss	Filter compression is inadequate (torque or hydraulic pressure).	If the holder is ours, ensure torque and hydraulic set point are correct. If the holder is from a third-party supplier, contact Technical Service for guidance on the appropriate torque settings.
	Membrane retention.	Ensure the correct membrane is used.
		Ensure there is no chemical incompatibility with membrane during process or cleaning.
High pressure drop	Feed channel is blocked.	Run cleaning in reverse direction (retentate to feed). Prefilter the feed stock and keep environment clean.
	There is an abnormally high pressure drop late in the process.	Ensure that the appropriate membrane screen and feed flow rate are selected.
	Devices are overtorqued.	Ensure devices are torqued to specifications. Loosen and retorque if necessary.
NWP issue	A procedural error has occurred.	Verify procedure and rerun test ensuring flows, temperature and pressures are stable prior to taking measurements (see Measurement of Normalized Water Permeability (NWP)).
	The system and device are not completely flushed.	Reflush system and device.
	The system and device are not completely cleaned.	Reclean system and device. Reevaluate cleaning procedure and solution (see Flushing and Cleaning).
Lines are visible through a maxi cassette jacket	The interface is misinterpreted as cracks.	Contact Technical Support.
Pellicon® 2 jacket becomes discolored	The color change is due to the oxidization of the urethane jacket.	Contact Technical Support.

Accessories and Spare Parts

Description	Quantity	Catalog Number
 Holders 		
Pellicon® 2 mini cassette holder (shipped with standard tie rod)	1	XX42PMINI
Pellicon® 2 holder, acrylic (shipped with short tie rod)	1	XX42P0060
Pellicon® 2 holder, stainless steel (shipped with long tie rod)	1	XX42P0080
 Tie Rods 		
Tie rod kit, standard, stainless steel, for mini holders	2	XX42PMIST
Tie rod kit, long, stainless steel, for mini holders	2	XX42PMILG
Tie rod kit, short, stainless steel, for acrylic holders	4	XX42P60TS
Tie rod kit, long, stainless steel, for acrylic holders	4	XX42P60TL
Tie rod kit, short, stainless steel, for stainless steel holders	4	XX42TIEST
Tie rod kit, long, stainless steel, for stainless steel holders	4	XX42TIELG
Cogent M1 mini holder long tie rods	2	CMP1423
 Spacers and Support Plate 		
Spacers for mini cassette holder, stainless steel	4	XX42PMSP
Spacers for benchtop holders, stainless steel	4	XX4200066
Support plate, stainless steel	2	PEH0TP2P1
 Gaskets 		
Gasket, silicone, no alignment tabs (for mini holders only)	5	FTP60056
Gasket with alignment tabs (for benchtop holders only)	10	PSSP00C10
Gasket with alignment tabs	10	XX42PEG10
Gasket with alignment tabs, double thick	10	PSSP2XC10
 Torque Wrenches 		
Torque wrench for Pellicon® 2 mini holder	1	XX42PMITW
Torque wrench for Pellicon® 2 benchtop holder installation	1	YY2029336
 Sockets 		
Socket, $\frac{9}{16}$ inch for Pellicon® 2 mini holder	1	XX42PMISR
Socket, $\frac{15}{16}$ inch for Pellicon® 2 benchtop holders	1	XX4200061
 Nuts and Washers 		
Nuts and washers ($\frac{5}{8}$ inch Nitronic® hex nuts and stainless steel washers) for benchtop holders	4	XX42P0069
Nuts and washers ($\frac{5}{8}$ inch silicone bronze hex nuts and stainless steel washers) for benchtop holders	4	XX4200079
Nuts and washers (hex silicone bronze nuts and washers)	4	XX42MT073

Standard Warranty

The applicable warranty for the products listed in this publication may be found at: www.millipore.com/terms (within the “Terms and Conditions of Sale” applicable to your purchase transaction).

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