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**1.0 Purpose**

This SOP describes the proper care and use of Cassette Filter Tangential-Flow Filtration (TFF) Systems, such as Millipore Pellicon™ – 2, Millipore Pellicon™ – 2 Mini-holder, and Pall Centrasette™.

**2.0 Scope**

Biopharmaceutical Development Program (BDP) Manufacturing personnel will follow this SOP.

**3.0 Authority and Responsibility**

3.1 The Manager of Purification, BDP has the authority to define this procedure.

3.2 BDP personnel are responsible for the implementation of this procedure.

3.3 Biopharmaceutical Quality Assurance (BQA) is responsible for quality oversight of this procedure.

**4.0 Materials and Equipment**

4.1 (WFI) Water For Injection (BDP PN 46213 or Freshly Drawn).

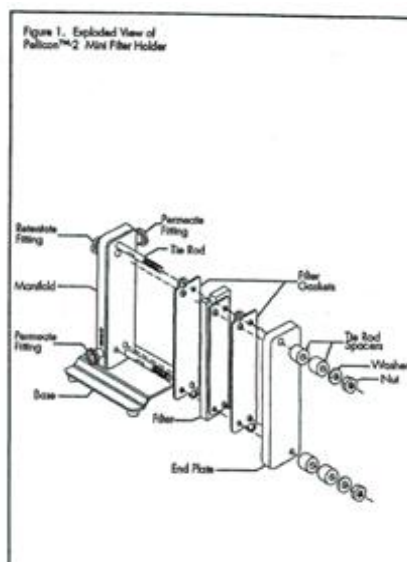
- 4.2 0.5N NaOH (PN-46109).
- 4.3 20% Ethanol (PN-46202).
- 4.4 Cassette Filter TFF Hardware (As appropriate for the process).
- 4.5 Millipore Cassette Filter(s).
- 4.6 pH Strips (BDP PN 20473 or BDP approved equivalent).
- 4.7 Watson-Marlow Pump (BDP MEF 68280 or equivalent).
- 4.8 Socket Wrench

### 5.0 Cleaning of Empty Cassette Filter Holder(s)

Ensure that the cassette filter holder(s) that are to be used have been cleaned and released per **SOP 14101, Cleaning of the Millipore Pellicon 2, the Millipore Pellicon 2 Mini Holder, and/or the Pall Centrasette TFF Holder Hardware**, and **SOP 21529 Equipment Interproduct Cleaning and Clearance**, before filter assembly.

### 6.0 Filter Cassette Installation (Pellicon™ – 2 Mini-holder hardware)

Figure 1



- 6.1 Remove the End Plate from the Manifold.
- 6.2 Ensure that all sealing surfaces of the manifold and end plate are clean and undamaged.
- 6.3 With WFI, wet the silicone filter gasket that is provided with the cassette. This will provide better adhesion to the manifold.

6.4 Holding the manifold with one hand, slide the filter gasket tabs over the tie rods and press the filter gasket against the manifold.

**NOTE:** The large feed and retentate holes on the filter gasket must align to the large feed and retentate holes on the manifold. Two filter gaskets are supplied with each filter. One filter gasket must be installed between each pair of filters, and one gasket must be installed between each end of the filter and the adjacent stainless steel plate.

6.5 Remove a filter cassette from its protective bag and examine the sealing surface thoroughly for any damage or foreign matter. Align the filter cut-outs with the tie rods and insert the filter so it is parallel with the manifold.

**NOTE:** The filter will extend beyond the edges of the manifold and end plate if the filter does not properly align to the feed, retentate, and permeate holes.

6.6 Holding the manifold with one hand, slide the filter gasket tabs over the tie rods and press the filter gaskets against the filter.

6.7 If you are adding a second or third filter, repeat Steps 6.5 – 6.6.

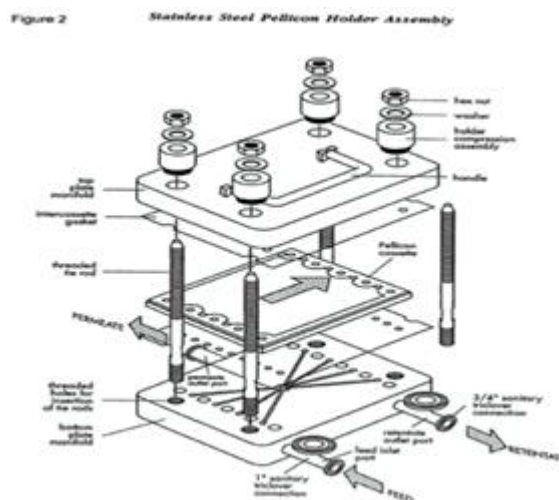
6.8 Holding the gaskets and filter(s) in place, with one hand, slide the end plate holes over the tie rods and press the end plate against the filter.

6.9 Replace in the following order, the tie rod spacers, then washers, and then nuts.

6.10 Hand tighten the nuts evenly by alternating from one nut to the other.

## 7.0 Filter Cassette Installation (Pall Centrasette™ and Pellicon™ – 2 Hardware,)

Figure 2



7.1 Remove the top plate manifold from the bottom plate manifold.

7.2 Visually inspect to ensure that all sealing surfaces of both manifolds are clean and undamaged.

- 7.3 With WFI, wet the silicone filter gasket that is provided with the cassette. This will provide better adhesion to the manifold.
- 7.4 Place the filter gasket flat on the bottom plate manifold. No special orientation is required; align the holes in the filter gasket with the manifold holes. Press the gasket against the manifold.  
**NOTE:** The filter cassette is supplied with two filter gaskets with each filter. One filter gasket must be installed between each pair of filters, and one gasket must be installed between each end of the filter and the adjacent stainless steel plate.
- 7.5 Remove a filter cassette from its protective bag and visually examine the sealing surface thoroughly for any damage or foreign matter.
- 7.6 Place the filter onto the silicone gasket. Orient the label on the filter cassette to the front of the holder, right-side up.
- 7.7 Place a filter gasket flat onto the installed filter cassette. Press the gasket against the filter. Repeat Steps 7.5 - 7.6 when mounting additional cassettes into the hardware.
- 7.8 Carefully install the top plate manifold with the handle side up.
- 7.9 Replace, in this order, the holder compression assemblies, washers, and nuts. For Pellicon™ holders with low membrane area, place spacers above the holder compression assembly so the nuts do not have to be screwed far down the tie rods to tighten the holder.
- 7.10 Hand-tighten the nuts in a diagonal fashion (See Figure 3).

## 8.0 Procedure to Tighten Pellicon™ – 2 Mini-holder Hardware

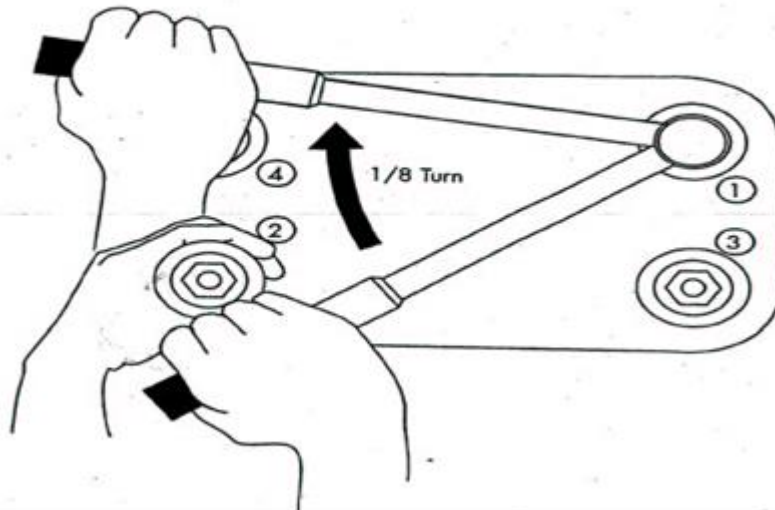
- 8.1 Using the pattern illustrated in Figure 3, tighten each nut in 1/8 turn increments using a socket wrench. Continue tightening the nuts using the proper tightening sequence. Repeat the tightening sequence three times to ensure even application of pressure on the filter(s).
- 8.2 Wait 5 – 10 minutes and retighten each nut.

**NOTE:** Waiting allows the gasket(s) to relax before retightening. The assembly must be retightened when the operating temperature differs from the tightening temperature, specifically if the operating temperature is lower than the tightening temperature.

**WARNING:** **DO NOT over tighten the nuts. Damage to cassettes may occur. Non-uniform tightening of the nuts can damage the filter. Non-parallel plates or compression of the filter(s) at one end can cause leakage.**

Figure 3

*Holder Tightening Sequence*



8.3 After sufficient tightening run WFI through the filter assembly. Observe the assembly for any leaking. If any leakage is observed, tighten the hardware until the leaking stops.

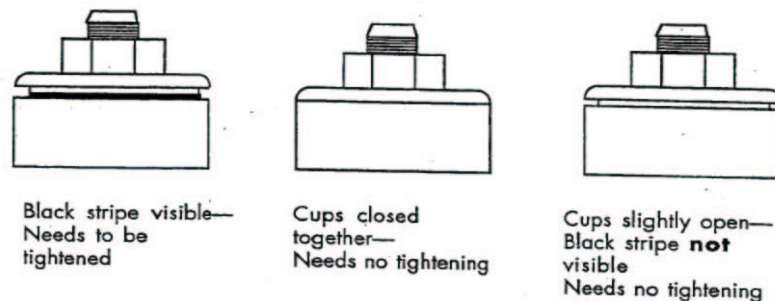
### 9.0 Procedure to Tighten Pall Centrasette™ and Pellicon™ – 2 Hardware

9.1 Turn each nut on the filter holder no more than  $\frac{1}{8}$  turn at a time, in the sequence shown in Figure 3.

9.2 Compress the holder compression assemblies until the white plastic “cups” close together. At this time, the black stripe will not be visible. If the black stripe reappears after some period of operation, retighten the nuts until the white cups close together. Retightening is not necessary until the black stripe reappears (See Figure 4).

Figure 4

*When to Tighten Holder Compression Assemblies*

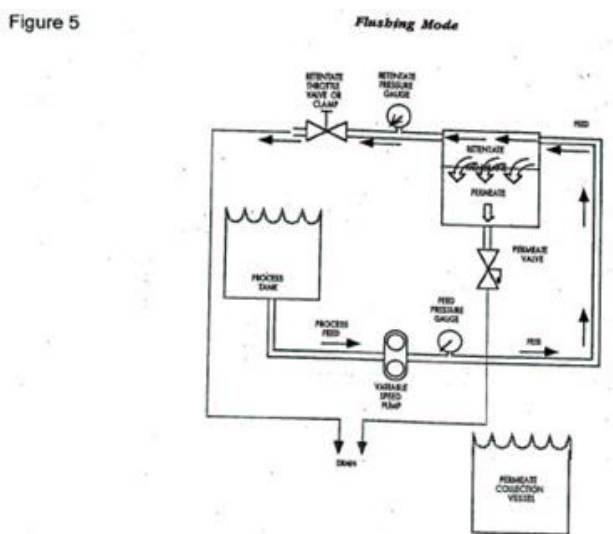


## 10.0 Preparation of Cassette and Hardware System

### 10.1 Flushing Procedure

**NOTE:** The Cassette Filters must be flushed prior to initial use to ensure removal of the cassette preservative. Also, perform flushing before buffer conditioning to remove chemical agents introduced during the storage process.

10.1.1 Set up TFF system as depicted in Figure 5 with WFI in the process tank/bag.



10.1.2 Fully open the retentate and permeate valves. Direct the retentate and permeate lines to drain.

10.1.3 Turn the pump on and increase the pump speed to obtain the following recommended Feed flow rate or Feed port pressure:

Pall Centrasette Cassettes: 1-2 liters per minute

Millipore Pellicon -2 Cassettes:

Type A Screen\* approximately 10 - 15 psi

Type C Screen\* approximately 5 - 10 psi

Type V Screen\* approximately 0.5 – 2.0 psi

(\* The letter indicating the screen type is the sixth digit in the catalog number printed on the edge of every Pellicon- 2™ filter.)

10.1.4 Flush the retentate side of the filter(s) until a minimum of 12 liters of water per m<sup>2</sup> of installed filter area has been removed from the retentate port.

- 10.1.5 Partially close the retentate valve and flush the filter(s) thru the permeate. Adjust the pump speed until the flow of WFI from the retentate port is approximately 1 - 2 liters per m<sup>2</sup> of installed filter area.
- 10.1.6 Flush the filter(s) until a minimum of 70 liters of water per m<sup>2</sup> of installed area has been removed from the permeate port.

**NOTE:** During the flushing of the retentate side of the filter some volume will flow out of the permeate port as well. The permeate volume removed during this step is unimportant and will vary according to the membrane type installed.

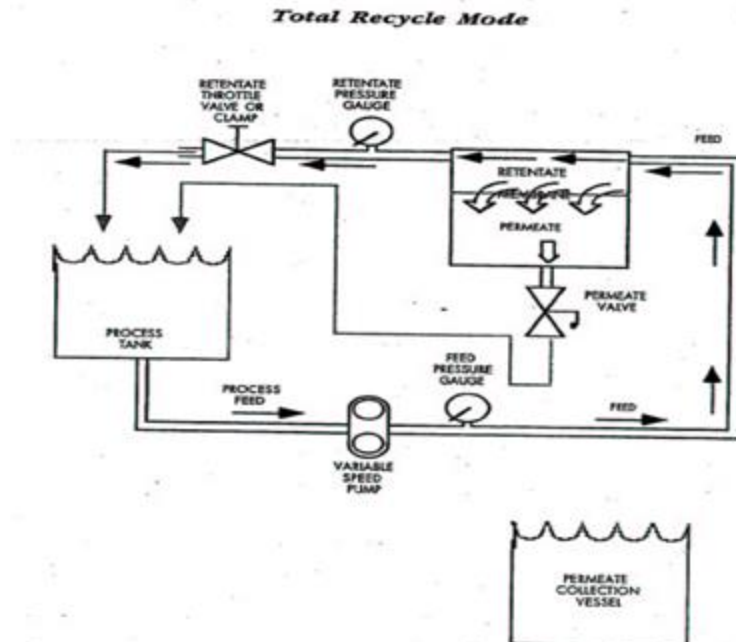
- 10.1.7 Verify that the pH of the retentate and permeate streams are pH 5 – 7 using pH strips.

**NOTE:** The two critical factors for effectively flushing the cassette and assembly are: (1) high cross-flow velocity to reduce boundary layer effects; and (2) positive transmembrane pressure profile along entire path length to assure adequate fluid transport through the membrane.

- 10.1.8 If pH has not returned to the neutral range, repeat Steps 10.1.2 to 10.1.7
- 10.2 Endotoxin Reduction Procedure

- 10.2.1 Direct the retentate and permeate lines back to the process tank as shown in Figure 6.

Figure 6





- 10.2.2 Open the retentate and permeate valves.
- 10.2.3 Set the pump speed to deliver the minimum amount of flow.
- 10.2.4 Fill the process tank/bag with 4-5 L of the appropriate solution. The following solutions and conditions are recommended for cleaning and endotoxin reduction of Filter Cassettes.

<u>Membrane</u>	<u>Solution</u>	<u>Conc.</u>
PT series	NaOH	0.5N
PL series	NaOH	0.5N
PHSA series	NaOH	0.5N
Durapore™	NaOH	0.5N
Pall Omega/Supor	NaOH	0.5N

- 10.2.5 Turn the pump on and increase the pump speed to obtain the following recommended Feed flow rate or Feed port pressure:

Pall Centrasette Cassettes: 1-2 liters per minute

Millipore Pellicon -2 Cassettes:

Type A Screen\* approximately 10 - 15 psi

Type C Screen\* approximately 5 - 10 psi

Type V Screen\* approximately 0.5 – 2.0 psi

(\* The letter indicating the screen type is the sixth digit in the catalog number printed on the edge of every Pellicon- 2™ filter.)

- 10.2.6 Using the retentate valve, adjust the retentate pressure to approximately 5 psi.
- 10.2.7 The following approximate pressure conditions should be observed.
  - Feed inlet pressure 10 - 30 psi.
  - Retentate outlet pressure 5 psi.
- 10.2.8 Recirculate the solution for  $\geq 60$  minutes.
- 10.2.9 After the process is complete turn off the pump and drain the system.
- 10.2.10 Remove endotoxin reducing solution and replace with a container of WFI. Direct retentate and permeate lines to drain.
- 10.2.11 Flush the system with WFI, using the same parameters described in Steps 10.2.5 – 10.2.7. Flush the filter(s) until a neutral pH has been achieved.



- 10.2.12 Verify that the pH of the retentate and permeate streams are pH 5 – 7 using pH strips.
- 10.2.13 If pH has not returned to the neutral range, repeat Step 8.2.11
- 10.2.14 When pH is in range, collect a sample of the effluent through the permeate and retentate lines and submit to PA for LAL release testing (Specification:  $\leq 0.5$  EU/mL).
- 10.2.15** Label the equipment per ***SOP 14150 Labeling of cGMP Purification Equipment for Cleaning Status.***

## 11.0 Storage of Membrane Cassettes

**NOTE:** The objective for proper storage of the membrane cassettes is to ensure the membranes remain wet, maintain their membrane properties, and prevent microbial growth during the time the cassettes are not in use.

- 11.1 Direct permeate and retentate lines back to the process tank (see Figure 6).
- 11.2 Open the retentate and permeate valves.
- 11.3 Set the pump speed to deliver the minimum amount of flow.
- 11.4 Add the 20% Ethanol to the process tank for storage.
- 11.5 Turn on the pump and set the pump speed to the following approximate Feed flow rate or Feed port pressure:
  - Pall Centrasette Cassettes: 1-2 liters per minute
  - Millipore Pellicon -2 Cassettes:
    - Type A Screen\* approximately 10 - 15 psi
    - Type C Screen\* approximately 5 - 10 psi
    - Type V Screen\* approximately 0.5 – 2.0 psi(\* The letter indicating the screen type is the sixth digit in the catalog number printed on the edge of every Pellicon- 2™ filter.)
- 11.6 Using the retentate valve, adjust the retentate pressure to approximately 5 psi. The following approximate pressure conditions should be observed.
  - Feed inlet pressure                      25 - 30 psi.
  - Retentate outlet pressure              5 psi.
- 11.7 Allow 4-5L of the storage solution to be collected through the filter.
- 11.8 Once the required amount of storage solution has been collected, close the system and label with a Yellow “Cleaned: Quarantined (Not Released for Use)” tag.

11.9 If Pall Centrasette™ filter(s) are to be stored for  $\leq 21$  days (or  $\leq 14$  days if using Millipore Pellicon™ filters) before use, the filters may be stored in the system at room temperature. Simply shut off the pump and clamp the lines to/from the holder or shut the feed, retentate, and permeate valves to keep the holder and filters full of storage solution. On Form 14125-01, note the expiration date for Pall Centrasette™ filter(s) as 21 days (or 14 days if using Millipore Pellicon™ filters) from the date of assembly.

11.9.1 If Pall Centrasette™ filter(s) are to be stored for  $> 21$  days (or  $> 14$  days if using Millipore Pellicon™ filters), seal the holder outlets and store the holder with the filters at  $2^{\circ}$ - $8^{\circ}$ C. On form 14125-01, N/A the expiration date adding the note "Filter stored at  $2^{\circ}$ - $8^{\circ}$ C."

**NOTE:** Pall Centrasette™ filters that are held for  $> 21$  days (or  $> 14$  days if using Millipore Pellicon™ filters) at room temperature must be flushed and cleaned as per Section 8.0, and a new sample submitted for LAL. If the results meet specification, store the filter as per Section 9.0. If the results do not meet specifications, the filter(s) must be flushed and recleaned prior to use.

## 12.0 Release of the Filter Assemblies

12.1 Verify that the LAL release data meets specifications,  $\leq 0.5$  EU/mL, and label the equipment per **SOP 14150 Labeling of cGMP Purification Equipment for Cleaning Status**.

12.2 If the release data does not meet specifications, repeat Sections 10.0.

## 13.0 Buffer Conditioning

**NOTE:** Buffer conditioning of the membrane cassettes and the entire TFF assembly is very important and critical for most biopharm applications. It assures that all the conditions listed below are achieved in the membrane cassette and in the feed, retentate, and permeate streams of the assembly, i.e., all surfaces in contact with the product.

- pH stability
- Ionic consistency
- Temperature stability
- Air and gas removed from fluid stream

The buffer used to condition the system must be at the same temperature as the process fluid. Membrane cassettes and hardware that experience a substantial temperature change ( $> 10^{\circ}$ C) will require a torque adjustment to be made on the cassette holder after a steady state is reached. For example, if the hardware surface temperature is  $20^{\circ}$  –  $25^{\circ}$ C and fluid is introduced at  $2^{\circ}$ - $8^{\circ}$ C, the torque on a full stack of cassettes may drop 20% - 40% due to contraction of the polymeric membrane elements.

**NOTE:** Before conditioning with the required buffer, be sure to flush the storage solution from cassette filters as per Step 10.2.11

- 13.1 Open the retentate and permeate valves.
- 13.2 Fill the process tank with enough buffer to completely flood the entire TFF assembly.
- 13.3 Set the pump speed to deliver the minimum amount of flow.
- 13.4 Turn on the pump. Adjust to the following conditions.

Feed inlet pressure	10 psi.
Retentate outlet pressure	5 psi.
- 13.5 Allow a minimum of 1-2 L of the buffer to be collected through the filter for conditioning.
- 13.6 Turn the pump off.
- 13.7 Record details of buffer conditioning in the project specific Batch Production Record (BPR).

#### 14.0 Filtration

**NOTE:** Trans Membrane Pressure (TMP) and cross flow rates must be optimized experimentally for each set of product conditions in order to achieve the best membrane performance. Information may be obtained from Millipore Technical Service to aide in this process.

- 14.1 Record details of sampling filtration in the project specific MPR.
- 14.2 Place the feed, retentate, and permeate lines in appropriate vessels and close the permeate valve. Turn on the pump and slowly begin pumping the sample through the cassette(s). This will minimize foaming.
- 14.3 Maintain a recirculation rate until all the air is removed from the system. Gradually increase the pump speed until the recommended recirculating flow rate is attained as indicated below.

##### **Recommended Tangential Flow Rate**

###### Standard Applications

"A" screen*	4 - 9 liters/minute/m <sup>2</sup>
"C" screen	9.5 - 14.6 liters/minute/m <sup>2</sup>
"V" screen	19.6 - 33 liters/minute/m <sup>2</sup>

\*The letter indicating the screen type is the sixth digit in the catalog number printed on the edge of every Pellicon-2 filter.

- 14.3.1 If needed, provide back pressure to the process by restricting the retentate flow. This can be done by slowly turning the retentate valve toward the off position until the desired pressure is attained.
- 14.4 Open the permeate valve slowly. This will generate the permeate flow and lower both the feed and retentate pressures and the recirculation flow rates.



- 14.4.1 To correct for the lowered recirculation flow rate, increase the speed of the pump. Adjust the back pressure accordingly.
- 14.4.2 Maintain the recommended recirculation rates to effectively minimize concentration polarization on the membrane surface.
- 14.5 When processing is complete, disassemble and discard used cassette filters and clean cassette holder hardware per **SOP 14101 Cleaning of the Millipore Pellicon 2, Millipore Pellicon 2 Mini-Holder, and/or the Pall Centrasette TFF Holder Hardware.**

### 15.0 Documentation

- 15.1 Maintain a separate logbook in the manufacturing area for each of the cassette filter systems. The logbook will contain chronological entries for all activity pertaining to each unit including cleaning, processing, maintenance and repair, storage, etc., as per **SOP 21531, Equipment/Facility Logbooks.**
- 15.2 Each unit must have a unique number for proper identification.
- 15.3 Document all activities (flushing, endotoxin reduction, storage, etc.) associated with cassette filters in their project specific BPR's (where applicable).

### 16.0 References and Related Documents

- 16.1 **Form 14125-01** Cassette Holder Filter Assembly, Endotoxin Reduction and Testing
- 16.2 **Form 14125-02** Cassette Filter TFF Assembly Use Log

### 17.0 Change Summary

