



# Steam-in-Place Method in the Reverse Direction for Millipore Express® PES Filters

## Introduction

The intent of this brief is to provide a Standard Operating Procedure (SOP) for steam sterilizing and integrity testing of Millipore's Express PES filters.

**Because a filter is vulnerable to damage during heat sterilization, it is recommended to test the integrity of sterilizing-grade filters after sterilization but before the filtration process.**<sup>1,2,3</sup> This SOP includes recommendations for steam sterilizing a product filter and conducting a pre-use integrity test, post steam sterilization.

The operations described in this SOP should be performed in the given sequence:

1. Reverse SIP of the hydrophilic sterilizing grade filter assembly used for the sterile filtration of the product (product filter).
2. Pre-use integrity testing of the product filter post steam sterilization.

**Note:** *The recommended post-SIP, pre-use filter integrity test for hydrophilic product filters is either the Enhanced Bubble Point test, which consists of both an air/water diffusion test and a bubble point test (SHF, SHC), or an air/water diffusion test (SHF, SHF, SHR). The pressure necessary for measuring the air/water bubble point of Millipore Express SHR membrane exceeds the pressure rating of the device. Integrity testing involves wetting the filter with a suitable wetting medium. System design must allow for post SIP, pre-use filter integrity testing without compromising the downstream sterility of the system.*

There are two options to assure adequate filter wetting and testing while maintaining sterility:

**Option 1:** If the downstream of the filter is attached to a vented sterile holding tank, the product can be used as a wetting agent. **Note:** *Absence of back pressure during the test is guaranteed by the large volume downstream (vented). For this option, a product-specific integrity test value will have to be determined and validated.*

**Option 2:** Use a sterilized vented collection vessel or other sterile barrier, such as the Millidisk® Barrier filter, to drain the downstream side of the product filter. This will allow you to use WFI to flush the line, remove extractables, wet the filter and ensure atmospheric pressure conditions downstream while also maintaining product line sterility.

There are two variations to this procedure:

- **Option 2A:** Steam-in-place the collection vessel. (No aseptic connection is needed if this option is used. This option allows the system to be steamed-in-place with the product filter.)

- **Option 2B:** Utilize a Millidisk Barrier filter. This option allows the system to be steamed-in-place with the product filter.

The Millidisk Barrier filter contains both hydrophilic and hydrophobic sterilizing-grade Durapore® membrane in one filter unit which allows for steam penetration, condensate removal, filter drying, extractables flush, filter wetting, and filter integrity testing without compromising the sterility of the steam sterilized system while maintaining atmospheric conditions to assure a valid test (refer to Millipore Millidisk Barrier filter data sheet PF1400EN00).

### Filter Characteristics

Millipore Express PES filters are sterilizing grade filters that are constructed with hydrophilic polyethersulfone membranes and polypropylene and polysulfone filter components. These filters are validated to withstand up to 3x steam-in-place (SIP) cycles at 135 °C for 30 minutes in the reverse direction. The maximum differential pressure allowed during SIP in the reverse direction is 69 mbar (1 psid).

### Steam-in-Place Procedure for Product Filter

The operations described in this SOP are designed for manual valve manipulations and should be performed in the given sequence.

**Note:** For automated systems, the drain valves are replaced by ON/OFF valves and automatic steam traps.

Prior to beginning the SIP procedure, the following is assumed:

- All connections are checked for proper fitting
- System has been checked for leak-tightness by means of a pressure hold test
- Product filter is completely dry prior to SIP
- All valves are closed and silicone tubing is attached to bleed valves and directed to a condensate drain
- Use caution to avoid contact with steam or hot stainless steel surfaces
- Wear protective glasses at all times and heat resistant protective gloves when necessary

## SIP Parameters

<b>Maximum SIP Temperature</b>	135 °C
<b>Maximum Reverse Differential Pressure During Steaming</b>	69 mbar (1 psid)
<b>Maximum Forward Differential Pressure with Air Across the Filter During Cooling</b>	345 mbar (5 psid)
<b>Cooling Time</b>	30 minutes (approximately)

## Integrity Testing Parameters

### Air/Water Diffusion

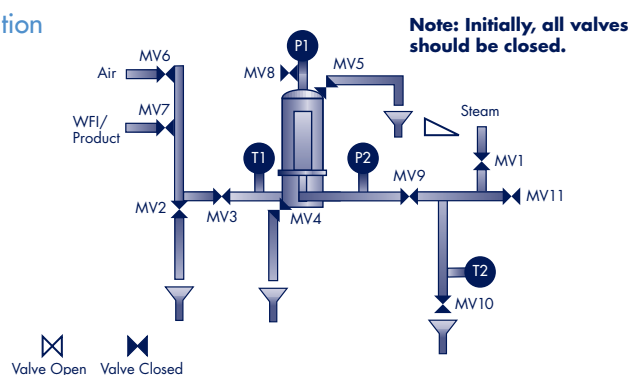
Millipore Express SHF Cartridge:	≤ 30 cc/min at 2.76 bar (40 psig) per 10-inch cartridge
Millipore Express SHC Cartridge:	≤ 28.2 cc/min at 2.76 bar (40 psig) per 10-inch cartridge
Millipore Express SHR with Prefilter:	≤ 27.1 cc/min at 3.45 bar (50 psig) per 10-inch cartridge

<b>Air/Water Bubble Point (SHF and SHC only)</b>	≥ 4.0 bar (58 psid)
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## Option 1

### Recommended Setup

#### Default Position



## Standard Operating Procedure: Option 1

**Note:** To begin, all valves are in the default closed position.

1. Check that the steam supply and compressed gas pressures are set at the required values.
2. Half open **MV10**.
3. Slowly open **MV1**. Purge the steam line until complete absence of condensate.
4. Slowly open **MV9** to progressively introduce steam and slowly heat the filter.
5. Half open **MV4**, **MV5**, **MV3**, and **MV2** to establish a flow path for air and condensate evacuation.
6. Partially close bleed valves **MV4**, **MV5**, **MV10**, and **MV2** so that a wisp of steam and continuous drops of water can be seen exiting.

**Note:** It is critical to control the differential pressure across the filter by controlling and monitoring the pressure difference between the gauges **P2** and **P1**. During steam procedure, always keep the delta-P across the filter at or under 69 mbar (1 psid) by adjusting backpressure with **MV4** and **MV5** when opened.

7. Ensure all air and condensate are effectively removed by keeping **MV4**, **MV5**, **MV10**, and **MV2** cracked open.

8. When the temperature downstream of the product filter, as measured by the temperature gauge **T1**, reaches the validated sterilization temperature parameters, the timing for sterilization is started.

**Note:** Millipore recommends a maximum of 3x SIP sterilization cycles at 135 °C for 30 minutes in the reverse direction. During the sterilization phase both pressure and temperature should be continuously monitored and recorded regularly.

9. Following completion of your validated sterilization cycle, close the steam supply valve **MV1** and immediately begin to [slowly] open **MV6** to introduce compressed sterile gas into the system. **MV3** should be fully opened to ensure adequate air flow. **CAUTION:** Verify that the system remains under positive pressure (as indicated by pressure gauges **P1** and **P2**). Control the air flow to ensure that the delta-P across the filter does not exceed 345 mbar (5 psid).
10. Allow steam to purge from all bleed valves and close valves **MV2** and **MV4** to increase the flow of gas through the system.

11. Ensure **MV5** is partially closed. Maintain sterile gas flow to cool down the system until the temperature gauge **T2** indicates approximately 30 °C.
12. Close valve **MV10** and **MV5**.
13. Keep **MV6**, **MV3**, and **MV9** open to maintain a positive pressure into the sterile filtration system while it is not in use.

## Post SIP, Pre-Use Filter Integrity Test Procedure: Option 1

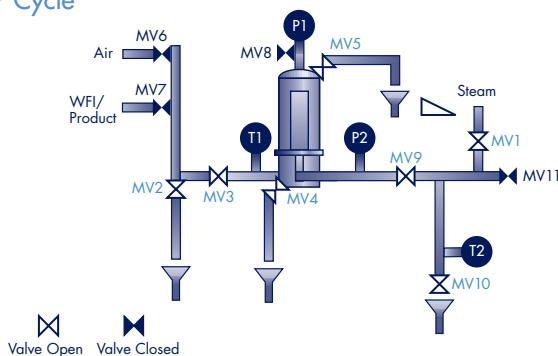
**Note:** For comprehensive filter wetting guidelines see Document P88057, *Wetting Procedures for Millipore Express PES filters*.

1. Open **MV11** if wetting fluid can be directed to the downstream equipment.
2. Ensure that the downstream of the product filter is vented and is at atmospheric pressure.
3. Ensure **MV3** is fully open. Close the compressed sterile gas supply valve **MV6**, and open **MV5** to vent the system. Wait for the pressure at **P1** and **P2** to drop to atmospheric pressure.
4. If possible, set the inlet pressure to 2.76 bar (40 psig). Gradually open **MV7** to fill the housing with wetting fluid. Vent the filter housing from **MV5**, and release all upstream air.

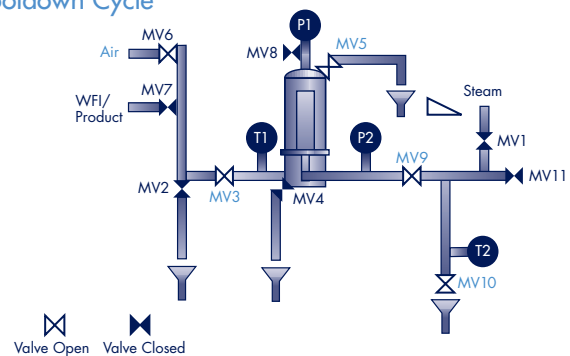
### Option 1

## Recommended SIP and Cooling Procedures

### SIP Cycle



### Cooldown Cycle



- When wetting fluid is seen exiting the top vent through the hose attached to **MV5**, close the vent valve **MV5** and continue to filter the wetting fluid into the downstream equipment (**MV11** flow path).
- Close **MV9** and maintain 2.76 bar (40 psig) pressure for at least one minute to dissolve residual gas within the filter and ensure membrane wetting.
- Fully open the downstream valve **MV9** and gradually open **MV11** to set the differential pressure (**P1 – P2**). (See **Table 1: Filter Wetting Guidelines**)
- Continue to flow wetting fluid at a flow rate of 1 L/min/ft<sup>2</sup> of membrane through the filter into the downstream sterile tank for at least five minutes.
- Close **MV7** to stop WFI/product flow.
- Fully open **MV11**.
- Once system reaches atmospheric pressure, attach an automatic filter integrity tester to **MV8** and fully open **MV8**.
- Verify that **MV8**, **MV9** and **MV11** are open.
- Close **MV3** and verify **MV4** and **MV2** are fully closed.
- Run the Enhanced Bubble Point test (SHF, SHC), or a diffusion test (SHF, SHC, SHR). The Enhanced Bubble Point test consists of both an air/water diffusion test and a Bubble Point test.
- When the test is finished and a positive result (i.e. pass) is obtained, close **MV8** and detach the filter integrity tester. The filter is now ready to use.

### Standard Operating Procedure: Option 2A

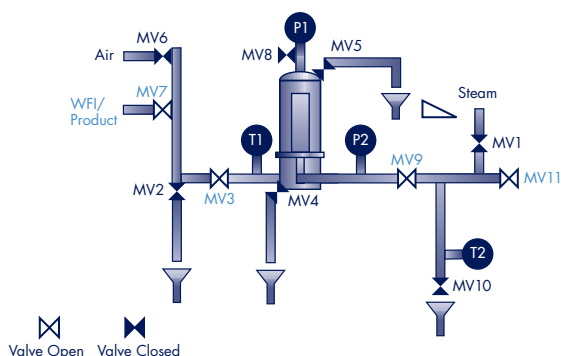
**Note:** To begin, all valves are in the default closed position.

- Check that the steam supply and compressed gas pressures are set at the required values.
- Open **MV10** and half open **MV16**.
- Slowly open **MV1**. Purge the steam line until complete absence of condensate.

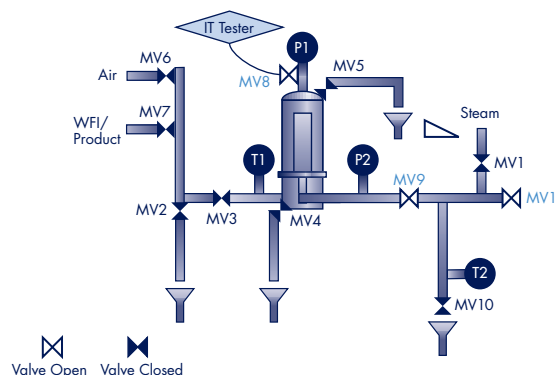
### Option 1

## Recommended Post SIP and Pre-Use Filter Integrity Test Procedures

### Wetting into Product Tank



### Enhanced Bubble Point Test/Diffusion Test



**Table 1**  
**Filter Wetting Guidelines**

Cartridge	Size		Area		Pressure		Approximate Flow Rate (L/min)
	cm	Inch	m <sup>2</sup>	ft <sup>2</sup>	mbar	psig	
Millipore Express SHF	13	5	0.29	3.1	53	0.77	3.1
Millipore Express SHF	25	10	0.54	5.8	57	0.82	5.8
Millipore Express SHF	50	20	1.08	11.6	57	0.82	11.6
Millipore Express SHF	75	30	1.62	17.4	57	0.82	17.4
Millipore Express SHC	13	5	0.23	2.5	72	1.05	2.5
Millipore Express SHC	25	10	0.50	5.3	68	0.99	5.3
Millipore Express SHC	50	20	0.99	10.6	68	0.99	10.6
Millipore Express SHC	75	30	1.48	15.9	68	0.99	15.9
Millipore Express SHR with prefilter	13	5	0.23	2.5	105	1.5	2.5
Millipore Express SHR with prefilter	25	10	0.50	5.3	95	1.4	6
Millipore Express SHR with prefilter	50	20	0.99	10.6	95	1.4	12
Millipore Express SHR with prefilter	75	30	1.48	15.9	95	1.4	18

4. Slowly open **MV9** to progressively introduce steam and slowly heat the filter.
5. Half open **MV2, MV4, MV5, MV3, MV13 and MV14** to establish a flow path for air and condensate evacuation.
6. Fully open **MV15**.
7. Partially close bleed valves **MV2, MV4, MV5, MV13, MV14, and MV16** so that a wisp of steam and a continuous drip of condensate can be seen exiting.

**Note:** It is critical to control the differential pressure across the filter by controlling and monitoring the pressure difference between the gauges **P2** and **P1**. During steam procedure, always keep the delta-P across the filter at or under 69 mbar (1 psid) by adjusting backpressure with **MV4** and **MV5** when opened.

8. Ensure all air and condensate are effectively removed by keeping **MV2, MV4, MV5, MV13, MV14, and MV16** cracked open.
9. When the temperature downstream of the product filter, as measured by the temperature gauge **T1**, reaches the validated sterilization temperature parameters, the timing for sterilization is started.

**Note:** Millipore recommends a maximum of 3x SIP sterilization cycles at 135 °C for 30 minutes in the reverse direction. During the sterilization phase both pressure and temperature should be continuously monitored and recorded regularly.

10. Following completion of your validated sterilization cycle, close the steam supply valve **MV1** and immediately begin slowly opening **MV6** to introduce compressed sterile gas into the system. **MV3** should be fully opened to ensure adequate air flow.

**CAUTION:** Verify that the system remains under positive pressure (as indicated by pressure gauges **P1** and **P2**). Control the air flow to ensure that the delta-P across the filter does not exceed 345 mbar (5 psid).

11. Allow the steam to purge from all bleed valves and close valves **MV2, MV4, MV14 and MV16** to increase the flow of gas through the system.
12. Ensure **MV5** is partially closed. Maintain the sterile gas flow to cool down the system until the temperature gauge **T2** indicates approximately 30 °C.
13. Close valve **MV13** and **MV5**.

14. Keep **MV6, MV3, MV9, and MV10** open to maintain a positive pressure into the sterile filtration system while it is not in use.

### Post SIP, Pre-Use Filter Integrity Test Procedure: Option 2A

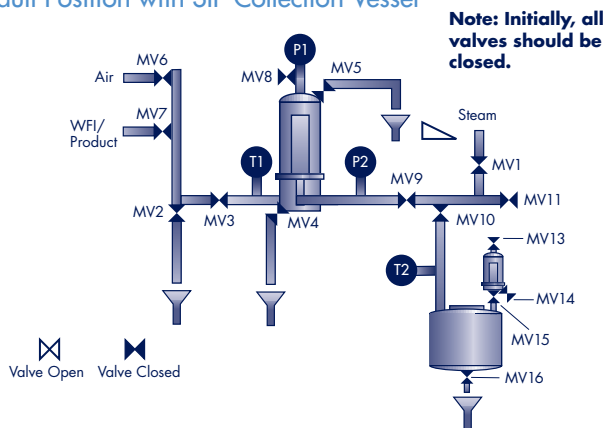
**Note:** For comprehensive filter wetting guidelines see Document P88057, *Wetting Procedures for Millipore Express PES Filters*.

1. If the wetting fluid is to be directed to a vented sterilized collection vessel open **MV10 flow path**. Ensure that **MV11** remains closed.
2. Ensure that the downstream of the product filter is vented and is at atmospheric pressure.
3. Maintain **MV3** open, close the compressed sterile gas supply valve **MV6**, and open **MV5** to vent the system.
4. Wait for the pressure at **P1** and **P2** to drop to atmospheric pressure.
5. If possible, set the inlet pressure to 2.76 bar (40 psig). Gradually open **MV7** to fill the housing with wetting fluid and vent the filter housing from **MV5**, until all upstream air has been released.

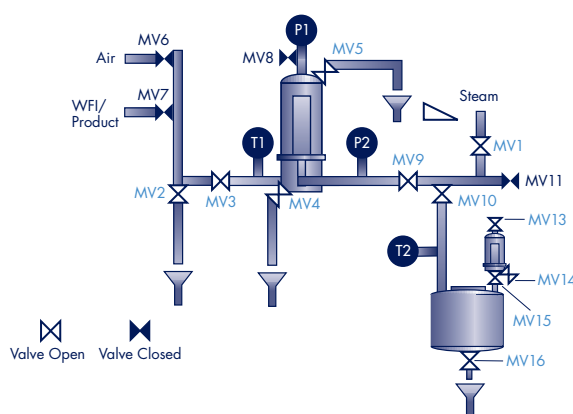
## Option 2A

### Recommended Setup and SIP Procedures

#### Default Position with SIP Collection Vessel



#### SIP Cycle



6. When wetting fluid is seen exiting the top vent through the hose attached to **MV5**, close the vent valve **MV5** and continue to filter the wetting fluid into the downstream equipment. Ensure **MV13** and **MV15** are fully open.
7. Close **MV9** and maintain 2.76 bar (40 psig) pressure for at least one minute to dissolve residual gas within the filter and ensure membrane wetting.
8. Fully open the downstream valve **MV9** and gradually open **MV10** to set the differential pressure (**P1 – P2**). (See **Table 1: Filter Wetting Guidelines**)
9. Continue to flow wetting fluid at a flow rate of 1 L/min/ft<sup>2</sup> of membrane through the filter into the sterile collection vessel for at least five minutes. (See **Table 1: Filter Wetting Guidelines**)
10. Close **MV7** to stop WFI/product flow.
11. Fully open **MV10**.
12. Once system reaches atmospheric pressure, attach an automatic filter integrity tester to **MV8** and fully open **MV8**.
13. Verify that **MV8**, **MV9**, **MV10**, **MV13** and **MV15** are open.
14. Close **MV3** and verify **MV4** and **MV2** are fully closed.
15. Run the Enhanced Bubble Point test (SHF, SHC), or a diffusion test (SHF, SHC, SHR). The Enhanced Bubble Point test consists of both an air/water diffusion test and a bubble point test.
16. When the test is finished and a positive result (i.e. pass) is obtained, close **MV8** and detach the filter integrity tester. The filter is now ready to use.

### Standard Operating Procedure: Option 2B

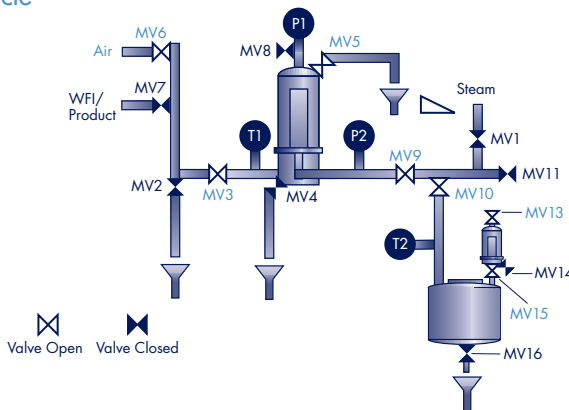
**Note:** To begin, all valves are in the default closed position.

1. Check that the steam supply and compressed gas pressures are set at the required values.
2. Open **MV10** and half open **MV13**.
3. Slowly open **MV1**. Purge the steam line until complete absence of condensate.
4. Slowly open **MV9** to progressively introduce steam and slowly heat the core of the filter.
5. Half open **MV2**, **MV4**, **MV5**, **MV3** and **MV12** to establish a flow path for air and condensate evacuation.
6. Partially close bleed valves **MV2**, **MV13**, **MV4**, **MV5** and **MV12** so that a wisp of steam and a continuous drip of condensate can be seen exiting.

### Option 2A

## Recommended Cooling Procedures

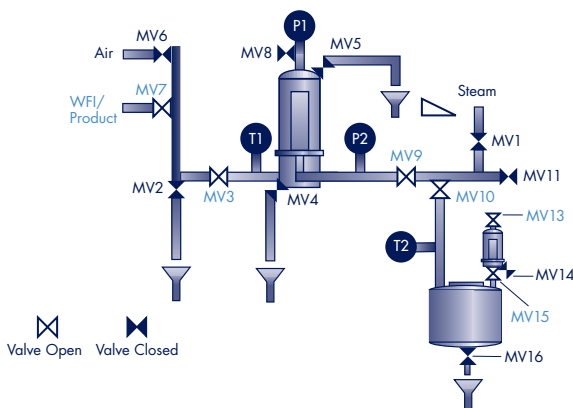
### Cooling Cycle



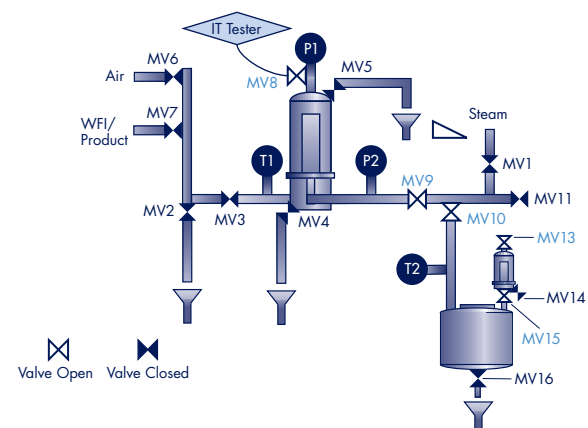
### Option 2A

## Recommended Post SIP and Pre-Use Filter Integrity Test Procedures

### Wetting into Collection Vessel



### Enhanced Bubble Point Test/Diffusion Test



**Note:** It is critical to control the differential pressure across the filter by controlling and monitoring the pressure difference between the gauges P2 and P1. During steam procedure, always keep the delta-P across the filter at or under 69 mbar (1 psid) by adjusting backpressure with MV4 and MV5 when opened.

7. Ensure all air and condensate are effectively removed by keeping MV2, MV13, MV4, MV5 and MV12 cracked open.
8. When the temperature downstream of the product filter, as measured by the temperature gauge T1, reaches the validated sterilization temperature parameters, the timing for sterilization is started.

**Note:** Millipore recommends a maximum of 3x SIP sterilization cycles at 135 °C for 30 minutes in the reverse direction. During the sterilization phase both pressure and temperature should be recorded regularly.

9. Following completion of your validated sterilization cycle, close the steam supply valve MV1 and immediately begin slowly opening MV6 to introduce compressed sterile gas into the system. MV3 should be fully opened to ensure adequate air flow.

**CAUTION:** Verify that the system remains under positive pressure (as indicated by pressure gauges P1 and P2). Control the air flow to ensure that the delta-P across the filter does not exceed 345 mbar (5 psid).

10. Allow the steam to purge from all bleed valves and close valves MV2, MV13, MV4 to increase the flow of gas through the system.
11. Ensure MV5 is partially closed. Maintain the sterile gas flow to cool down the system until the temperature gauge T2 indicates approximately 30 °C.
12. Close valve MV12 and MV5.
13. Keep MV6, MV3, MV9, and MV10 open to maintain a positive pressure into the sterile filtration system while it is not in use.

### Post SIP, Pre-Use Filter Integrity Test Procedure: Option 2B

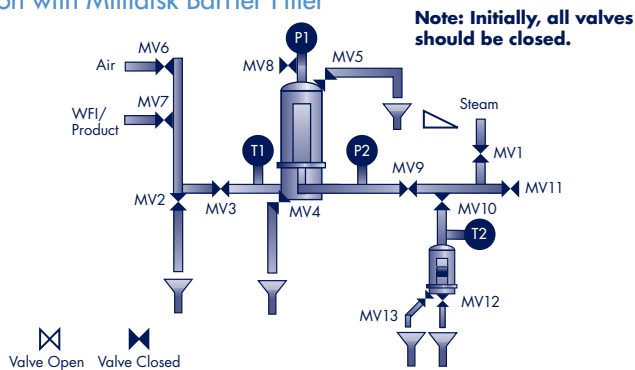
**Note:** For comprehensive filter wetting guidelines see Document P88057 Wetting Procedures for Millipore Express PES Filters.

1. Use MV10 flow path to direct wetting fluid to the Millidisk Barrier filter. Ensure that MV11 remains closed.
2. Ensure that MV10 and MV12 are open to a Millidisk Barrier filter.
3. Maintain MV3 open, close the compressed sterile gas supply valve MV6, and open MV5 to vent the system.
4. Wait for the pressure at P1 and P2 to drop to atmospheric pressure.
5. If possible, set the inlet pressure to 2.76 bar (40 psig). Gradually open MV7 to fill the housing with wetting fluid.

### Option 2B

## Recommended Setup

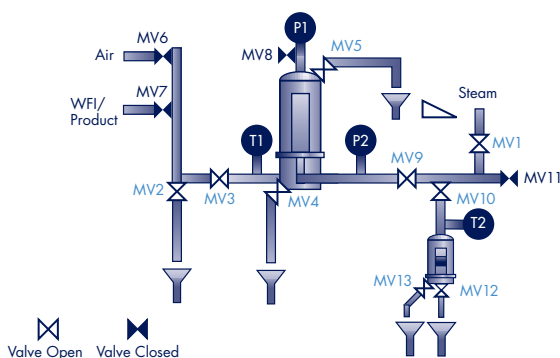
### Default Position with Millidisk Barrier Filter



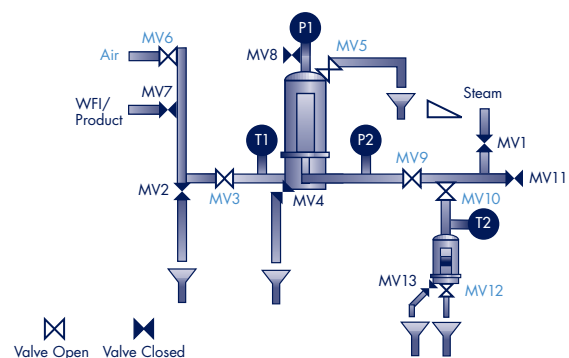
### Option 2B

## Recommended SIP and Cooling Procedures

### SIP Cycle with Millidisk Barrier Filter



### Cooling Cycle with Millidisk Barrier Filter



6. Vent the filter housing from **MV5**, to release all upstream air.
7. When wetting fluid is seen exiting the top vent through the hose attached to **MV5**, close the vent valve **MV5** and continue to filter the wetting fluid through the Millidisk Barrier Filter. Ensure **MV12** is fully open.
8. Close **MV9** and maintain 2.76 bar (40 psig) pressure for at least one minute to dissolve residual gas within the filter and ensure membrane wetting.
9. Fully open the downstream valve **MV9** and adjust **MV10** to set the differential pressure ( $P1 - P2$ ). (See **Table 1: Filter Wetting Guidelines**)
10. Continue to flow wetting fluid at a flow rate of 1 L/min/ft<sup>2</sup> of membrane through the filter into the drain via the barrier filter for at least five minutes. (See chart for filtration area)
11. Close **MV7** to stop WFI/product flow.
12. Fully open **MV10**.
13. Once system reaches atmospheric pressure, attach an automatic filter integrity tester to **MV8** and fully open **MV8**.
14. Verify that **MV8**, **MV9**, **MV10**, and **MV12** are open.
15. Close **MV3** and verify **MV4** and **MV2** are closed.
16. Run the Enhanced Bubble Point test (SHF, SHC), or a diffusion test (SHF, SHC, SHR). The Enhanced Bubble Point test consists of both an air/water diffusion test and a bubble point test.
17. When the test is finished and a positive result (i.e. pass) is obtained, close **MV8** and detach the filter integrity tester. The filter is now ready to use.

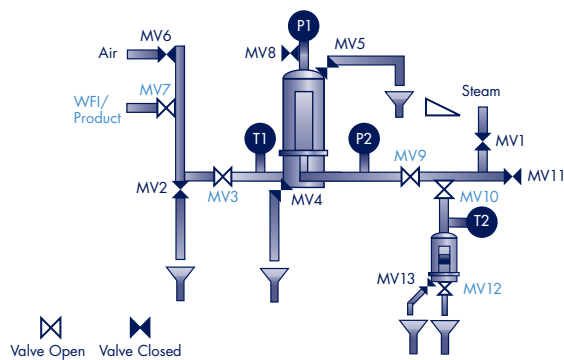
## References

1. **Revision of annex 1 to EC Guide to GMP for Sterile Medicinal Products**; 1997. "The integrity of the sterilized filter should be verified before use and should be confirmed immediately after use by an appropriate method such as a bubble point, diffusive flow or pressure hold test."
2. **Sept. 2004 Draft FDA Guideline on Sterile Drug Products Produced by Aseptic Processing** "Normally, integrity testing of the filter is performed after the filter apparatus has already been assembled and sterilized. It is important that integrity testing be conducted after filtration to detect any filter leaks or perforations that might have occurred during filtration. Forward flow, bubble point and pressure hold tests are acceptable integrity tests."
3. **PDA Technical Report No. 26, Sterilizing Filtration of Liquids**; 1998. "Integrity testing of sterilizing grade production filters pre and post use is a fundamental element of sterility."

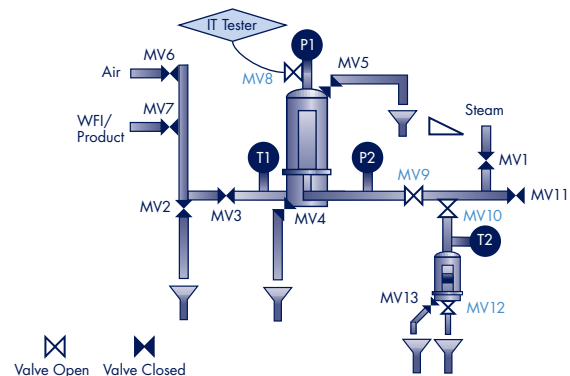
## Option 2B

### Recommended Post SIP and Pre-Use Filter Integrity Test

#### Wetting Through Millidisk Barrier Filter



#### Enhanced Bubble Point Test/Diffusion Test



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Lit. No. TB1750EN00 Rev. A 09/05 05-271  
Printed in U.S.A.

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