

Installation and
Operating
Instructions

Steamer Line
Hollow Fiber
Cartridges

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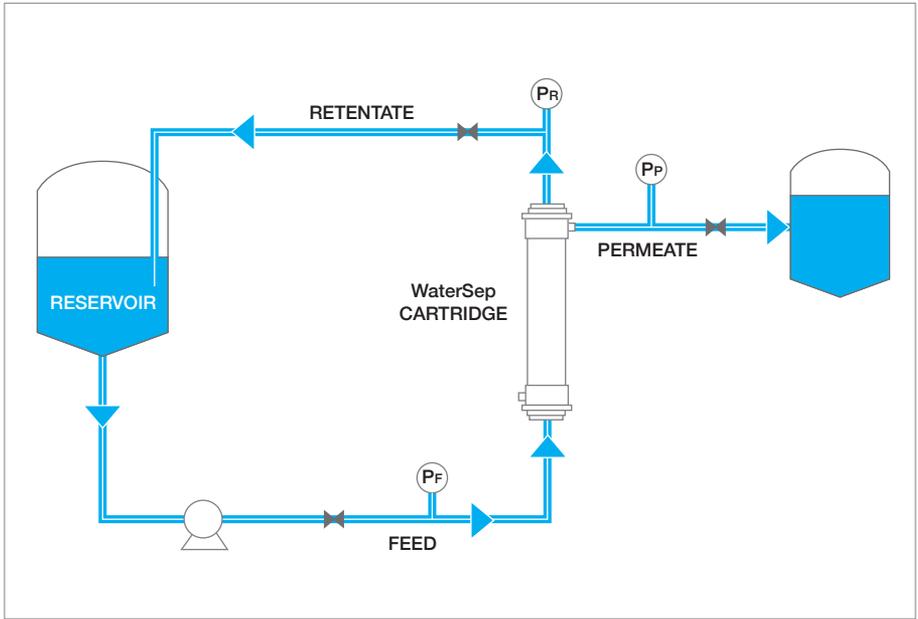


Designed for perfusion and aseptic applications, WaterSep's **Steamer Line** autoclavable hollow fiber cartridges are gamma irradiated and ready to use. They do not contain humectant and are pre-wetted with deionized water so minimal flushing is needed before use. The cartridges can be cleaned, stored and autoclaved up to five (5) times.

Contents:

1. Connect the **Steamer** cartridge into a crossflow system.
2. Autoclave the **Steamer** cartridge or system, if required for your application.
3. Measure the water flux and normalized water permeability if intended to reuse.
4. Integrity test the **Steamer** cartridge.
5. Condition the **Steamer** cartridge with buffer before introducing product.
6. Run the filtration process and recover your product.
7. Clean the **Steamer** cartridge if intended to reuse.
8. Store the **Steamer** cartridge.
9. Flush the **Steamer** cartridge to remove the storage solution.

Figure 1: Crossflow System



1. Connect the Steamer cartridge into a perfusion/crossflow system

Inspect the cartridge

On the bag containing the cartridge, check that the gamma label is red indicating that the cartridge was gamma irradiated. Open the bag and remove the caps and plugs from all the ports on the cartridge.

Connect the cartridge

Connect the cartridge to the crossflow system. Figure 1 shows a typical crossflow system with tubing, pump, valves and pressure sensors. The retentate and permeate should be connected to appropriate containers or a bioreactor.

Table 1: Operating Limits

WaterSep [®] BioSeparations	Maximum Feed Pressure		Maximum Transmembrane Pressure (TMP)		Maximum Operating Temperature		pH Range
	bar	psi	bar	psi	°C	°F	
0.45µm & 0.2µm	1.0	15	0.7	10	40	104	2 to 13.5
0.1µm	1.4	20	1.0	15	40	104	2 to 13.5
75K MWCO	1.7	25	1.4	20	50	122	2 to 13.5
500K - 300K MWCO	2.1	30	1.7	25	50	122	2 to 13.5
100K - 3K MWCO	2.8	40	2.4	35	60	140	2 to 13.5

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Re-Wet the cartridge (Optional)

New WaterSep Steamer Line hollow fiber cartridges are pre-wetted with deionized water or can be re-wetted with deionized water prior to autoclaving. The recommended re-wetting volume for new cartridges is 0.5L/m² through the permeate for 5 minutes. For rinsing storage solution from a used cartridge, refer to Step 9.

To re-wet the cartridge and system, fill the reservoir with water. Start the feed pump at a slow speed. Use a pinch valve on the retentate to generate backpressure and transmembrane pressure (TMP) to get permeate flow through the fibers. Slowly increase the pump speed so the TMP = 0.5 – 10 psig to wet the cartridge and remove air from the system. TMP is calculated using the equation:

$$\text{TMP} = \{(\text{P feed} + \text{P retentate}) / 2\} - \text{P permeate}$$

Direct the retentate and permeate to waste. During flushing, adjust the retentate backpressure. After the cartridge and system is re-wetted, decrease the feed pump speed then stop the feed pump and discard the waste.

2. Autoclave the Steamer cartridge or system, if required for your application

The cartridge can be autoclaved connected to a system. Plastic sanitary clamps should be loose for autoclaving. The cartridge should be autoclaved using a liquid or wet cycle for 35 minutes at 121°C (maximum); do not use a dry or solid autoclave cycle. Once the autoclave cycle is complete, allow the cartridge to cool to ≤50°C degrees prior to handling. The sanitary clamps can be re-tightened after the cartridge has cooled to ≤50°C degrees. WaterSep Steamer Line cartridges can be autoclaved up to five (5) times.

3. Measure water flux and normalized water permeability if intended to reuse

If the cartridge is going to be cleaned and reused, we recommend to perform a normalized water permeability before use as a reference. With the retentate valve open and the permeate valve closed, fill the reservoir with water. Start the feed pump at a slow speed. Slowly increase the pump speed so the feed pressure is 10psi for ultrafiltration cartridges and 5psi for microfiltration cartridges. Rinse the cartridge with clean water for 10 minutes or until the rinsing water flows from the retentate and permeate ports.

(Water) Flux

Open the permeate valve and adjust the retentate valve so the TMP is = 0.5 – 5 psig of water. Measure and record the following data: 1) permeate flow rate in mL/min; 2) feed pressure in psi; 3) retentate pressure in psi; 4) permeate pressure in psi; and 5) permeate temperature in degrees Celsius. Flux, with units of Liters per square meter per hour (LMH), is calculated using the equation:

$$\frac{\text{Flow Rate} \left(\frac{\text{mL}}{\text{min}} \right)}{\text{Surface Area} (\text{m}^2)} \times 0.06$$

Normalized Water Permeability (NWP)

Water permeability is typically performed with water temperature of 20°C and is calculated using the equation:

$$\text{Water Permeability at } 20^{\circ}\text{C} = \frac{\text{Flux (LMH)}}{\text{TMP (psig)}}$$

If the actual water temperature for the permeability test is not 20°C, the water permeability calculated above should be normalized to 20°C with a temperature correction factor (TCF) from Table 2. The Normalized Water Permeability is calculated using the equation:

$$\text{Normalized Water Permeability (NWP)} = \frac{\text{Flux (LMH)}}{\text{TMP (psig)}} \times \text{TCF}$$

Figure 2: Process Diagram for Pressure Hold Integrity Test

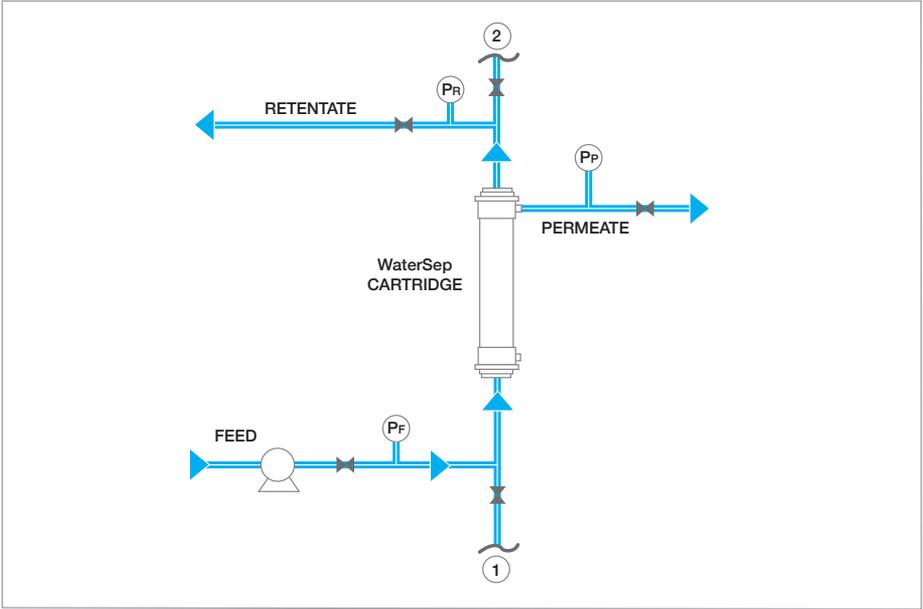


Table 2: Temperature Correction Factors for Water Temperatures from 5°C — 50°C

Actual Water Temperature (°C)	5	10	15	20	25	30	35	40	45	50
TCF	1.520	1.308	1.139	1.002	0.891	0.798	0.720	0.653	0.596	0.547

4. Integrity test the Steamer cartridge (refer to Figure 2)

- a. Circulate water through the hollow fiber cartridge for 10 minutes. Apply transmembrane pressure to ensure fluid flows from both permeate ports.
- b. Attach an external pressurized air source to the feed/retentate side of the hollow fiber system using either location ① or ② in Figure 2.
- c. Close the permeate valve. Open retentate valve and feed port.
- d. Pressurize the system slowly to the specified pressure:
 - 10psi for UF membranes
 - 5psi for MF membranes
- e. Drain the system of any liquid upstream.
- f. Close the retentate valve.
- g. Open the permeate valve.
- h. Let the system equilibrate for 5 minutes, while allowing any remaining liquid to pass through the HF cartridge.
- i. Close the feed port and monitor the pressure decay in the system.
- j. The system/cartridge is integral if the pressure gauge reads a positive upstream pressure after 1 minute.

CAUTION: Use only white plastic (nylon) clamps on cartridges with sanitary fittings.

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5. Condition the **Steamer** cartridge with buffer before introducing product

Fill the reservoir with buffer that is the same buffer as the product being filtered. Start the feed pump at a slow speed. Slowly increase the pump speed so the TMP = 0.5 – 5 psig to remove air and flush water from the system. Recirculate the buffer in the cartridge for several minutes until the pH equilibrates.

6. Run the perfusion or crossflow filtration process and recover your product

PERFUSION PROCESS

Connect the perfusion system to the bioreactor and begin perfusing based on the conditions for your application. Perfusion rates can range from 0.2 vessel volumes per day (VVD)—2 VVD or more, depending on your procedure. After processing the cartridge can be cleaned for reuse or discarded.

CROSSFLOW PROCESS

Set your flow rate

Connect or add the process feed to the reservoir. Route the retentate and permeate tubing to the appropriate containers for your process. Close the permeate valve and start the feed pump at a slow speed to recirculate the process fluid. Slowly increase the pump speed and open the permeate valve to achieve the crossflow feed and permeate flow rate for your cartridge per the Flow Rates on Table 4. If needed, use the retentate valve to change the retentate backpressure to achieve the permeate flow rate for your cartridge.

Choose your crossflow filtration operating mode

The operating mode you choose will depend on your application and filtration conditions. **TMP control** is typically used with ultrafiltration membranes. For **TMP control**, the user sets the crossflow rate and uses the retentate valve to adjust the backpressure and initial TMP. During **TMP control**, adjust the retentate back pressure to be less than the maximum Operating Limits listed on Table 1 while allowing the flux to vary during the process.

Permeate flow control processing is often used with microfiltration for cell harvesting and cell clarification to control the flow through the more open pores. **Permeate flow control processing** uses a permeate pump to maintain a relatively constant flux while allowing the TMP to vary during the process.

A typical TMP range for a concentration/diafiltration process is 10–30psi (0.7–2Bar) and for a cell harvest/clarification process is 3 – 10psi (0.2–0.7Bar). Pressures observed during filtration should not exceed the maximum Operating Limits (Table 1).

For optimal product recovery at the end of processing, close the permeate valve and release the retentate backpressure. Recirculate the fluid for 5–10 minutes to allow product to re-enter the bulk solution flow.

Pressures observed during filtration should not exceed the maximum Operating Limits (Table 1). Once you have recovered your retentate or permeate product, then stop the pump(s). Disconnect the product.

7. Clean the **Steamer** cartridge

The **Steamer** cartridge can be cleaned and autoclaved after each use up to five (5) times. The choice of cleaning solution, cleaning time and frequency will depend on the specific process stream, fouling tendency and operating conditions.

Rinse the cartridge

After use, rinse the cartridge with 5–10L/m² of clean water or buffer. Close the permeate ports and allow the rinsing solution to go to drain.

Clean the cartridge

Suggested chemical cleaning reagents are listed in Table 3 and can be warmed up to 40°C. Direct the retentate

Clean the cartridge (continued)

and permeate lines to the feed reservoir. Close the permeate valve. Use a slow crossflow rate and no retentate backpressure to clean the feed/retentate. Recirculate the cleaning solution for 60 – 90 minutes, then open the permeate valve and clean the permeate. Confirm the cleaning solution flows from the permeate port. Alternatively, clean the feed/retentate with fresh cleaning reagent, discard the reagent used for cleaning the feed/retentate, then use fresh cleaning reagent to clean the permeate.

Table 3: Suggested chemical cleaning reagents (Note: Do not exceed a maximum temperature of 40°C)

0.5N – 1.0N Sodium Hydroxide
0.5N – 1.0N Sodium Hydroxide + 400ppm Sodium Hypochlorite
Surfactants & Detergent with or without enzymes* *Use manufacturer's recommended concentration.

Rinse and post-use membrane recovery

After cleaning, open the permeate and retentate valves. The rinsing solution will go to the drain. Rinse the cartridge with clean water until the pH of the permeate and retentate equilibrate to the same pH as the clean water.

Integrity test the cartridge

After cleaning and rinsing test the cartridge to confirm integrity, as you did in Step 4.

Measure water flux

Measure the flux, as you did in Step 3, using clean water to confirm the cartridge has been cleaned. Using the same flow conditions as for the pre-use flux, the post-use water flux of a cleaned cartridge should be within 80% of the flux when the cartridge was new and preferably greater than 90%.

Table 4: Cartridge Model Length, Membrane Surface Area and Sample/Batch Volume

Cartridge Model	Discover™			Explorer™		
	12"	24"	41"	12"	24"	41"
Cartridge Length (in)	12"	24"	41"	12"	24"	41"
Surface Area (m ²)	0.0052	0.0107	0.019	0.0155	0.032	0.058
Sample/Batch Volume	10 – 850mL			150 – 3,000mL		

Fiber Id	Recommended Crossflow					
0.5 mm ID	0.036			0.11		
1.0 mm ID	0.14			0.43		
2.0 mm ID	0.58			1.7		

Note: The crossflow pump rates are to achieve a shear rate of ~4000 sec⁻¹. To achieve a shear rate of ~6000 sec⁻¹ multiply the pump rate (L/m²h)

LMH		Permeate					
30	Ultrafiltration	0.16	0.32	0.58	0.47	1.0	1.7
60	Ultrafiltration	0.31	0.64	1.16	0.93	1.9	3.5
20	Microfiltration	0.10	0.21	0.39	0.31	0.64	1.2
40	Microfiltration	0.21	0.43	0.77	0.62	1.3	2.4

Note: The permeate flow rate for UF is 30 – 60 LMH and MF is 20 – 40 LMH. Process flow rates may vary depending on the feed composition

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8. Store the Steamer cartridge

Fill the reservoir with 0.1N NaOH. Open the permeate port, direct the retentate and permeate lines to the feed reservoir. Similar to conditioning a new cartridge, introduce the storage solution using a slow crossflow rate and no retentate backpressure. Recirculate the solution until the pH ≥ 9 from all outlet ports. Disconnect the cartridge and seal the cartridge's ports with caps or plugs, as appropriate. Store the cartridge at 4 – 25°C until needed.

9. Flush the Steamer cartridge to remove storage solution

A used Steamer cartridge stored in 0.1N NaOH solution needs to be flushed out prior to reusing the cartridge.

Connect the cartridge and wet the system

To fully flush the cartridge and system, fill the reservoir with water. Start the feed pump at a slow speed. Use a pinch valve on the retentate to generate backpressure and transmembrane pressure (TMP) to get permeate flow through the fibers. Slowly increase the pump speed so the feed and TMP = 0.5 – 10 psig to wet the cartridge and remove air from the system.

Flush the cartridge

The Recommended Flush Volume through the permeate: for Discover™ and Explorer™ cartridges is 80L/m², and for Investigator™ and larger cartridges is 40L/m² to flush the storage solution from the cartridge. Direct the retentate and permeate to waste. During flushing, adjust the retentate backpressure. Rinse the cartridge with clean water until the flushing water has reached neutral pH from the retentate and permeate ports, indicating the storage solution has been flushed from the cartridge. After the cartridge is flushed, decrease the feed pump speed then stop the feed pump and discard the waste. Note: warm water up to 40°C will facilitate flushing the storage solution from the cartridge.

Materials of Construction

The product as assembled is USP Class VI compliant.

Membrane: Modified Polyethersulfone (m-PES) **Housing:** Polysulfone **Encapsulant:** Medical Grade Epoxy

Researcher™			Investigator™			mini-BioProducer™			BioProducer™		
12"	24"	41"	12"	24"	41"	12"	24"	41"	12"	24"	41"
0.044	0.094	0.172	0.13	0.28	0.51	0.61	1.35	2.50	1.21	2.69	5.00
400 – 8,000mL			1 – 25L			5 – 250L			25 – 500L		

Pump Requirements (L/min)

0.33	1.0	4.8	9.6
1.3	4.0	19	38
5.2	16	77	154

(in) by a factor of 1.5. To achieve a shear rate of ~8000 sec⁻¹ multiply the pump rate (L/min) by a factor of 2.0.

Flow Rate (L/hr)

1.3	2.8	5.1	3.9	8.4	15	18	41	75	36	81	150
2.7	5.6	10	7.8	17	31	37	81	150	73	161	300
0.89	1.9	3.4	2.6	5.6	10	12	27	50	24	54	100
1.8	3.8	6.9	5.2	11	20	24	54	100	48	108	200

and viscosity, membrane, operating conditions such as TMP, crossflow rate and temperature.

WaterSep provides Hollow Fiber Crossflow Filtration from Concept to Completion. Our Products are Easy to Use and Tailored to Your Specific Filtration Application.

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FlowConnector™ Kits, FlowStands™ and FlowSystems – One Stop Shopping

WaterSep has the hardware you need to complement your cartridge. FlowConnector™ Kits connect your WaterSep cartridge to your tubing assembly, and our FlowStand™ secures your cartridge vertically with custom sized holders. Our FlowSystems include a console with an embedded PC, pumps, and feed flow and pressure control capabilities. Let our experts customize a FlowSystem to fit your application.

Additional WaterSep Product Lines

Single Use Green Line

For single use crossflow applications, WaterSep offers gamma irradiated, humectant free and ready to use Green Line products. The Green Line cartridges require no pre-use flushing and are offered as a single use FlowAssembly™.

ReUse Line

Designed for those applications where cleaning and reuse is acceptable, ReUse Line cartridges can be cleaned, rinsed, stored and used up to twenty (20x) in the same application.

For questions, contact us at:
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