Millipore®

User Guide

Natrix® CH Pilot and Process Chromatography Membrane

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Contents

Introduction

Natrix® CH Pilot and Process are manufacturing-scale chromatography devices containing a high-capacity cation exchange chromatography membrane. They are supplied ready to use and work with existing chromatography systems. Efficient in operating at fast flow rates, Natrix® CH is well suited for frontal or bind and elute applications and is ideal for rapid cycling operations. In addition, Natrix® CH can easily scale up from a laboratory to a clinical and commercial manufacturing scale. The conveniently stackable cassette device style maintains chromatographic efficiency at all scales.

This instruction guide applies only to Natrix® CH Pilot and Process chromatography membrane devices. For information on other single-use chromatography products visit www.sigmaaldrich.com.

Technical Information

Definitions

Membrane volume (MV) is the quantity of membrane available for binding within the device. MV is also used in this document to describe both fluid volumes and flow rates (in MV/min). The use of MV is analogous to the use of column volume (CV) in column chromatography.

Materials of Construction

Component	Material
Membrane	Polyacrylamide hydrogel reinforced with polybutyleneterephthalate substrate
Screen separator	Copolymer of polypropylene and polyethylene
Chemistry	Sulfonic acid and t-butyl
Housing	Copolymer of styrene and phenylene ether

Specifications

Parameter		Specification
Nominal Membrane Volume (mL)	Pilot	124
	Process	372
Membrane Configuration		Flat sheet
Membrane Bed Thickness (mm)		1.8
Typical Lysozyme Binding Capacity (mg/mL)*		90
Typical mAb binding capacity (mg/mL)*		80
Typical mAb loading capacity in Frontal mode (g/L) **		1000
Flow Rate Range (MV/min)		≤ 10
Maximum Operating Pressure (psi/bar)		75/5

*10% breakthrough dynamic binding capacity in 20 mM sodium phosphate buffer, pH 7.0.

**Loading capacity is not limited to 1 kg/L and depends on the feed stream composition and target aggregate removal.

Chemical Compatibility

Natrix® CH membrane is compatible with most aqueous buffers and solvents commonly used in biomolecule purification processes. Use this information only as a guide, since chemical compatibility can be influenced by conditions including exposure time, temperature, and chemical concentration.

Chemical
0.1 M HCl
1 M NaOH
20% Ethanol
2% Acetone
6 M Guanidine hydrochloride
8 M Urea

Storage and Handling

Natrix® CH Pilot and Process are supplied in dry condition. Natrix® CH Pilot and Process devices should be stored in the original packaging in a clean, dry location at room temperature and away from direct sunlight.

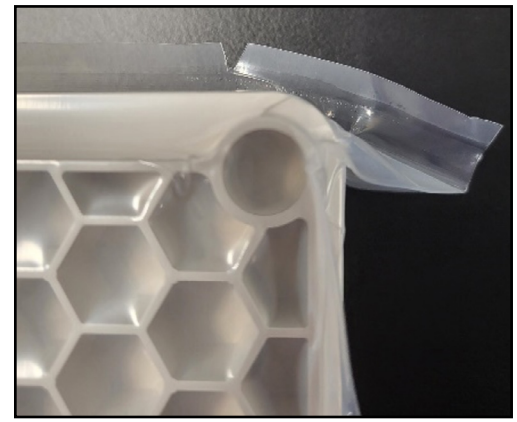
NOTE Do not freeze.

Operating Procedure

Preparation

Ensure the mAb feed and all buffers are sterile filtered with a 0.22 µm membrane before contacting with the Natrix® CH Pilot and Process devices. Before applying the mAb feed to a new device, ensure that it is equilibrated with the equilibration buffer. Optionally, run a blank cycle before applying sample. Please note that the devices are supplied double-bagged as dust covers.

NOTE There is a tear notch in the bag to facilitate opening.

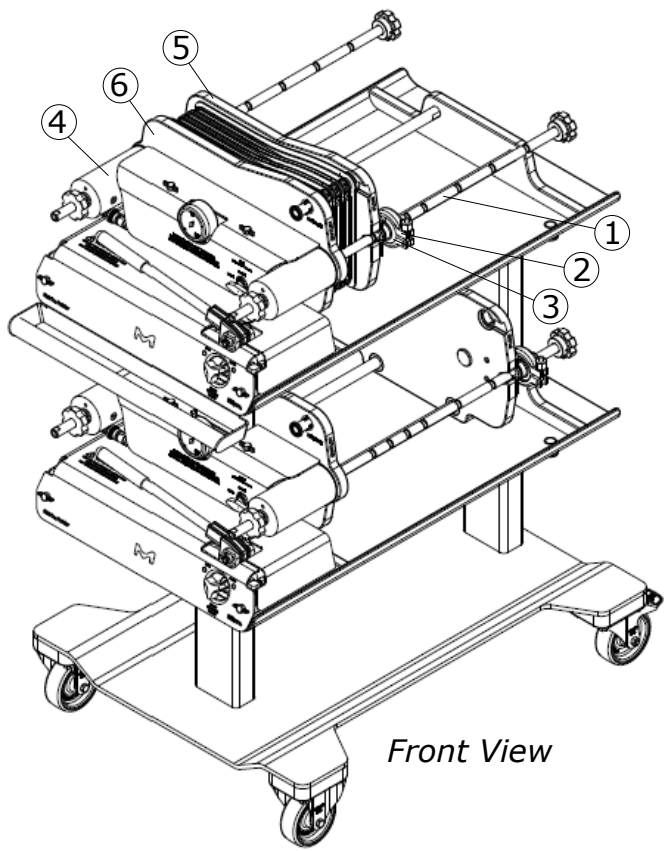


Determining the Blank Column Pressure

Determining the blank column pressure is important and it must be deducted from the delta column pressure to estimate the actual device delta column pressure.

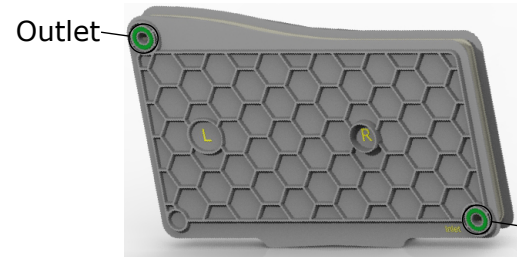
Select the column position which will be used to test the performance of the Natrix® CH Pilot or Process device. Connect the lines for this column position using a union connector. Change the flow rate to run at 10 MV/min. Once the pressure stabilizes, note the delta column pressure value. This value is the blank pressure of the system, which is needed to determine the actual operating delta column pressure of the device.

Installation



Front View

1. Natrix® CH Pilot and Process devices have a designated inlet and outlet:



Schematic of Natrix® CH Pilot or Process device.

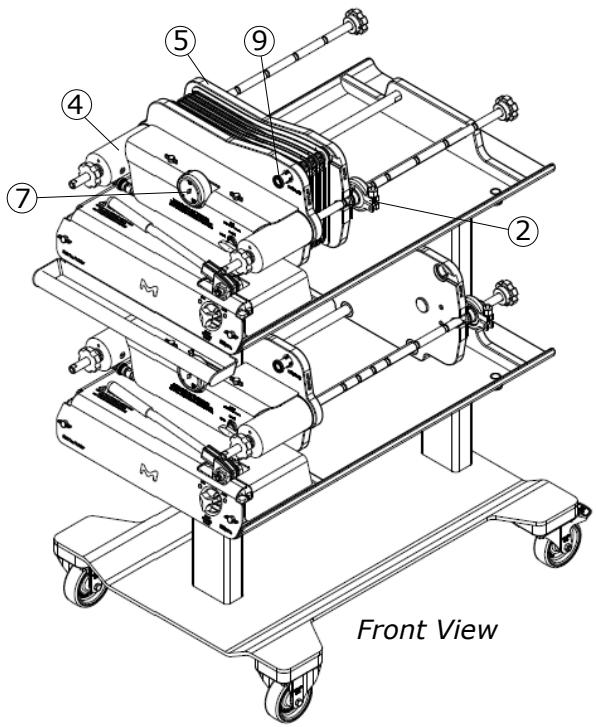
   Gaskets should be in place on the inlet and outlet ports.

2. Disposable fittings need to be installed on the process holder. The fittings are available under part number NXPF001PR0. Please refer to user guide for process scale holder for more details.

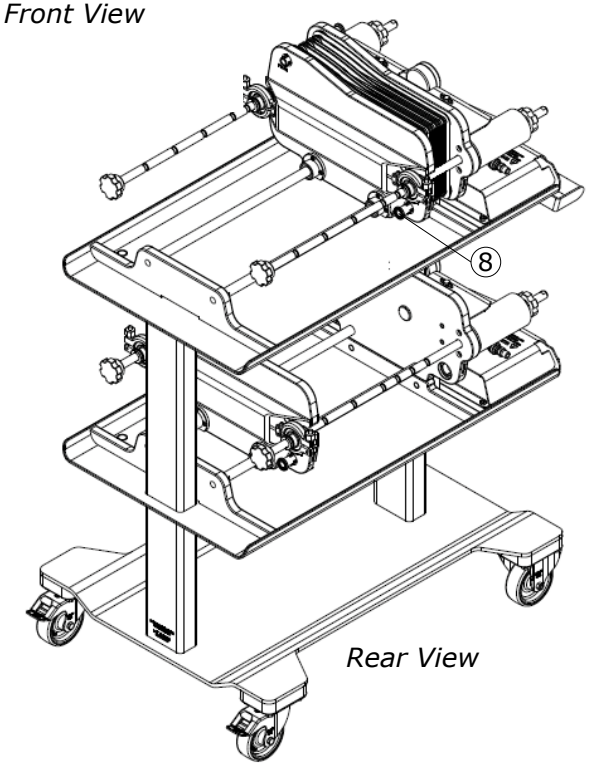
3. Install the device on the process holder. Remove the clamp rod ① from the side that the devices will be loaded by removing the clamp rod knob from one end and the tri-clover clamp ② and clamp insert ③ from the opposite end, then pulling the clamp rod through the hydraulic cylinder ④. The hole for the clamp rod in the movable endplate ⑤ is slotted, allowing the rod to be removed without fully withdrawing it from the holder.

4. Grasp the channel on the moveable endplate ⑤ and pull it open enough to install the devices. Gaskets are installed on the disposable fittings and must be in place for proper operation.

5. Position the device so that the alignment key of the device faces the fixed endplate ⑥ of the holder. **If the devices are installed backwards, the alignment key will prevent the devices from locking into each other or into the fixed endplate ⑥ and will cause damage to the devices when hydraulic pressure is applied**.



Front View



Rear View

6. Install devices from the side by gently lowering the device into the holder. The devices will self-align on the alignment rods. Push the first device against the fixed plate. Push each additional device against the next, ensuring that the alignment keys engage.

7. When all devices are installed, push the moveable endplate ⑤, in the center below the channel, against the last device, ensuring that the alignment keys engage.

8. Replace the clamp rod. Install the clamp inserts and tri-clover clamps ② into the clamp rod adjustment groove that is closest to the moveable endplate ⑤. Turn the clamp rod knobs until they contact the hydraulic cylinders. If the piston is not retracted to within 5 mm, continue turning the knob until the piston is retracted.

9. Close the hydraulic pump release valve. The hydraulic pump vent valve must be in the Vent position during operation. Open the hydraulic valve and increase the hydraulic pressure until the hydraulic pressure gauge ⑦ on the holder reads 900 - 1100 psi (62 - 76 bar). Do not increase the pressure if the hydraulic valve is closed.

10. Once approximately 1000 psi (69 bar) is achieved on the gauge, close the hydraulic valve. To ensure that the moveable endplate ⑤ will compress properly, the piston of hydraulic cylinder ④ should not extend more than 40 mm (1.6 in.) beyond the hydraulic cylinder. If it does, relieve the pressure in the hydraulic system, retract the pistons manually and repeat steps 7 through 10.

    NOTE A loss in hydraulic pressure due to settling of devices may occur if an assembly is compressed and left unused for an extended period. The minimum hydraulic pressure required to maintain an integral assembly is 800 psi. Should the hydraulic pressure be observed below 800 psi, repeat 10.

11. Connect the process piping to the sanitary fittings. The feed piping must be connected to the inlet port ⑧. The filtrate must be connected to the outlet port ⑨.

Priming

1. Prior to priming the device, prime the lines by using tri-clover connector and flowing buffers at 10 MV/min.
2. Stop the flow and remove the tri-clover connector.
3. Connect the device's inlet to the inlet tubing and outlet to the outlet tubing.
4. Start the buffer flow at 1 MV/min from inlet to outlet. Gradually increase it to 10 MV/min. This will remove any bubbles on the device.
5. When no more air is observed exiting the device (either visually with clear tubing or by UV signal), decrease the flow rate to 1 MV/min and reverse the flow.
6. Continue flowing equilibration buffer through the device at a flow rate of 1 MV/min until the UV, pH, pressure, and conductivity detectors have reached a constant value.
7. Change the flow direction to upflow and gradually increase the flow to 10 MV/min until UV, pH, pressure, and conductivity detectors have reached a constant value.

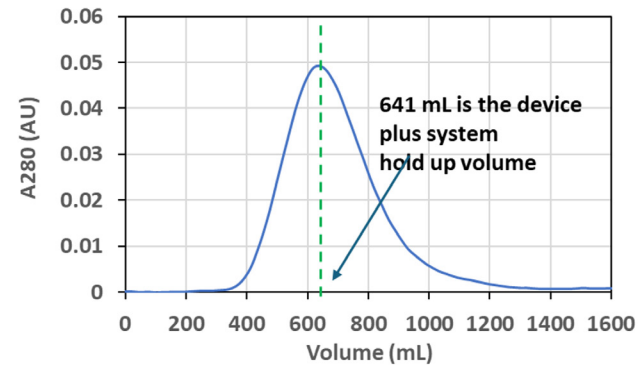
Sanitization

The device is not supplied sterile; therefore, proper sanitization is recommended before use. The recommended sanitization solution is 0.5 M NaOH for 30 min. After sanitization, flush the device with equilibration buffer until the pH and conductivity values stabilize in the expected range.

Hold-up Volume and System Integrity Test

The device integrity test is intended to determine if there are any gross defects in the device, though calculation of asymmetry or HETP is not necessary. We recommend using 2% acetone or 1 M NaCl in the equilibration buffer as the tracer solution. If the peak is split or otherwise severely distorted, the device might not be integral, and we recommend discontinuing use of the device and contacting Technical Services for a replacement. Integrity test for each device should be conducted separately.

1. To check the system integrity and estimate the hold-up volume, flow equilibration buffer at a flow rate of 10 MV/min through the device and monitor the UV280 or conductivity detector to establish a baseline signal.
2. Load 2% acetone or 1 M NaCl solution in equilibration buffer into the 125 mL (Pilot) or 370 mL (Process) injection loop.
3. Inject the tracer solution while continuing to flow equilibration buffer at a flow rate of 10 MV/min. The time/volume will be set to zero at this injection event.
4. The observed peak maximum volume is the system hold-up volume. An example of the chromatogram generated during the measurement of the system hold-up volume with a Natrix® CH Pilot device is shown below:



Example of a chromatogram showing UV_{280} of a 2% acetone pulse used to measure the system hold-up volume

Recommended Buffer Composition for Purification of mAb in Frontal Mode

Step		Buffer Solution	Volume (MV)	Flow rate (MV/min)
Number	Description			
1	**Equilibrate** with a buffer that has the same pH and conductivity as the mAb feed that will be processed.	Acetate or phosphate buffer is recommended.	10	≤ 10
2	**Load** the membrane with the feed.	Post protein A or protein A and anion exchange polished mAb feed pH adjusted 0.22 µm filtered.	Dependent on sample loading	≤ 10
3	**Wash** with the equilibration buffer to push through any feed that remains on the device.	Acetate or phosphate buffer is recommended.	10	≤ 10
4	**Strip 1** with 0.5 M NaCl to remove impurities that remain on the membrane.	0.5 M NaCl in acetate or phosphate buffer is recommended.	10	≤ 10
5	**Strip 2** with a 1M NaCl solution.	1M NaCl in acetate or phosphate buffer is recommended.	10 – 20	≤ 10
6	**Clean in place** (sanitize).	0.5 M sodium hydroxide + 50 mM NaCl.	10 – 20	≤ 10
7	**Strip 3**	1M NaCl in acetate or phosphate buffer is recommended.	10 – 20	≤ 10
8	**Re-equilibrate** for the next cycle.	Acetate or phosphate buffer is recommended.	10	≤ 10

NOTE The recommended Equilibration buffer or load pH and conductivity is 5.0 and 9 mS/cm, respectively. The pH and conductivity should be optimized for each feed.

Standard Product Warranty

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

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