Millipore®

User Guide

Natrix® CH Bench Chromatography Membrane

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Contents

Introduction

The Natrix® CH Bench is a medium-scale membrane chromatography device containing Natrix® CH membrane, a high-capacity cation exchange chromatography membrane. It is supplied ready to use and works 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. Additionally, Natrix® CH can easily scale up from a laboratory to a clinical and commercial manufacturing scale.

This instruction guide applies only to Natrix® CH Bench (8.8 mL) 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 to describe both fluid volumes and flow rates (in MV/min) in this document. 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)	8.8
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
Connections	5/16”-24 threads

*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 Bench is supplied in dry condition. Natrix® CH Bench 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 Bench device. 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. 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. The size of the connecting tubing affects the pressure and thus it is important to determine the blank column pressure.

Select the column position, which will be used to run the Natrix® CH Bench device. Connect the lines for this column position using a union connector. Change the flow rate to run at 88 mL/min in down-flow mode. 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

Natrix® CH Bench devices have threaded connections on both ports. Ensure connection to the inlet and outlet as marked on the device. The following shows the tube and fittings required for Natrix® CH Bench devices:

- Tubing ⅛-inch O.D.
- Flangeless male nut ⅛-inch, quantity 2
- Tubing connectors ⅛-inch, quantity 2
- PEEK threaded adapters ¼-28 x 10-32, quantity 2
- Flangeless Ferrules ⅛-inch, quantity 4

1. Connect the inlet and outlet with tube and threaded fittings:

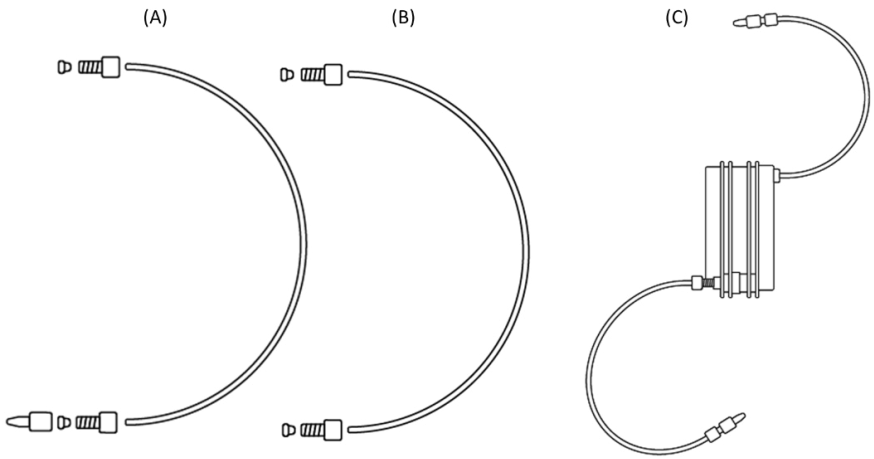

Figure 1: Connection of the tubing and fittings to the Natrix® CH Bench device

2. Orient the device so that the inlet port is located on the bottom-right corner of the device and the outlet port is located at the top-left corner when viewing from the inlet side. Flow equilibration buffer at 15 mL/min in the forward direction of the device (inlet to outlet) until buffer is observed discharging from the outlet and no air bubbles are present.
3. Flip the device upside-down so that the inlet port is now located at the top-left corner when viewing the device from the inlet side. Reverse the flow direction so that buffer is now flowing at 15 mL/min from outlet to inlet. This will remove any air bubbles on the inlet side of the device. Gently tapping the device may help to remove trapped air.
4. When no more air is observed exiting the device (either visually with clear tubing or by UV signal), change the flow back to the forward direction (inlet > outlet) and turn the device right side up.
5. Attach the device holder to the chromatography system and insert the device into the holder by sliding it into place.
6. Gradually increase the flow rate to 10 MV/min and monitor the pressure drop (DeltaC Pressure) across the device. Continue flowing at a rate of 10 MV/min until the pressure is stable. Gently tapping the device will help to remove any remaining air.
7. Continue flowing equilibration buffer through the device at a flow rate of 10 MV/min until the UV, pH, pressure, and conductivity detectors have reached a constant value.

Sanitization

The device may be sanitized prior to use if desired. The recommended sanitization solution is 0.5 M NaOH for 30 minutes. After sanitization, flush the device with equilibration buffer until the pH and conductivity values stabilize in the expected range. It is not recommended to store the device(s) wet.

Integrity Testing

Holdup 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 in the equilibration buffer as the tracer solution (alternatively, 1M NaCl can be used). If the peak is split or otherwise severely distorted, the device may not be integral, and it is recommended to discontinue the device preparation/ installation. Contact Technical Services for a replacement.

1. To check the system integrity and estimate the holdup 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 1M NaCl) solution in equilibration into the 1 mL 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 Bench device is shown below:

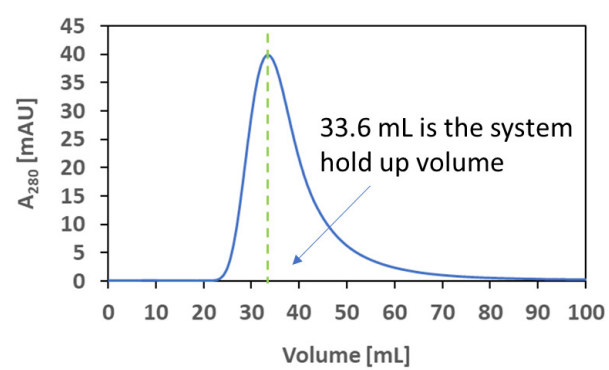

Figure 2: Example of a chromatogram showing UV280 of a 2% acetone pulse used to measure the system hold-up volume

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 mAb 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 pH and conductivity of equilibration and load depends on the optimum condition to maximize yield and impurity removal. Generally, pH 4 – 6 and 3 – 9 mS/cm conductivity is recommended.

Offline Integrity Testing

NOTE Natrix® CH Filters must be wetted prior to integrity testing.

Test the integrity of the filter by measuring the diffusional flow rate of air through the wetted membrane. Refer to the table below for the diffusion flow rate specification:

Device	Device Catalog Number	Air Diffusion Flow Rate at 20 psi (1.4 bar) in Water (sccm)
Natrix® CH Bench	NXCH001BEN	≤ 100

The integrity test can be conducted using either an automated integrity tester or a manual testing setup.

Automated Integrity Testing

The Integritest® Automated Integrity Test Instruments are recommended. To test Natrix® CH Bench devices effectively on the Integritest® system, the below test parameters are recommended:

Test Parameter	Specification
Diffusion Pressure Specification	20.0 psi
Diffusion Flowrate Specification	100 mL/min
Extended Diffusion	Yes
Extended Diffusion Time	2 minutes
Number of Filter Rounds	1
Custom Low Flow	0.10 mL/min
Custom Low Volume Limit	1 mL

Refer to the instrument user guide for instructions on creating custom protocols.

For other automated integrity testers, the auto or default stabilization time setting can stop the test prematurely, and the resulting equilibration time may not be sufficient to achieve an accurate air diffusion measurement. Contact Technical Service for guidance with other integrity testers.

1. Wet the device with deionized water at 40 psi for 2 minutes, or until air bubbles no longer appear in the outlet stream.
2. After wetting is complete, disconnect the device and gently shake it to remove any excess water.
3. Connect the device to the automated integrity tester, select the appropriate recipe, and start the test. Note that additional water may be expelled from the device outlet while the test is running.
4. If the device fails the integrity test, rewet the device, check the integrity test protocol used, and retest. If the device still fails after retesting, contact Technical Service for confirmation and defect analysis.

   NOTE If a *Gross Leak Detected* or *Max Pressure Loss Exceeded* error occurs, this could also be an indicator of device integrity loss. Check for leaks in connection and ensure device is properly wetted before retesting. If error persists, contact Technical Service.

Manual Integrity Testing

The integrity test may be performed manually using a mass flowmeter. Multiple measurement ranges may be required as flow values vary highly from integral devices (< 10 sccm) to nonintegral devices (> 100 sccm).

1. Wet the device with deionized water at 40 psi for 2 minutes, or until air bubbles no longer appear in the outlet stream.
2. After wetting is complete, disconnect the device and gently shake it to remove any excess water.
3. Apply dry air at 20 psi for 5 minutes, or until no liquid is visible coming from the outlet port.
4. Connect the device outlet to a manual flow meter. Run air through the device at 20 psi and allow it to stabilize for 2 minutes before recording a flow value.
5. If the device fails the integrity test, rewet the device and retest. If the device still fails after retesting, contact Technical Service for confirmation and defect analysis.

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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For technical assistance please visit:
www.sigma-aldrich.com

For additional information and documentation please contact:
Merck KGaA, Darmstadt, Germany
Corporation with General Partners
Frankfurter Str. 250
64293 Darmstadt, Germany
Phone: + 49 6151-72 0

For requests from USA and Canada please contact:
MilliporeSigma
A subsidiary of Merck KGaA, Darmstadt, Germany
400 Summit Drive
Burlington, MA 01803 USA
Phone: 1-800-645-5476

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