INSTRUCTION GUIDE

Natrix Recon HD-Sb

Hydrogel Membrane Devices

Natrix®

This product has been developed, manufactured, packaged and distributed under the strictest controls to ensure product quality, safety and consistency. Natrix Separations Inc operates in accordance with a Quality Management System that is certified compliant with ISO 9001:2008.

Membranes are tested for flow rate, dynamic binding capacity, and thickness. Finished adsorber tests include flow rate and dynamic binding capacity.

This product is intended for laboratory / research use and is not supplied sterile.

Read operating instructions carefully prior to use of Natrix products.

TABLE OF CONTENTS

Natrix

SECTION 1: INTRODUCTION

With a revolutionary three-dimensional macroporous hydrogel structure that provides a high density of binding sites and rapid mass transfer, Natrix HD Membranes deliver binding capacity that exceeds resin-based columns with fast flow rates typical of membrane adsorbers.

The Natrix HD-Sb membrane is a strong cation exchange membrane augmented with HIC groups. This high capacity and high throughput membrane is ideal for bind-and-elute purification of biomolecules such as monoclonal antibodies (mAb).

This instruction guide is for Natrix Recon HD-Sb devices only. For information on other Natrix products please visit www.natrixseparations.com.

SECTION 2: TECHNICAL INFORMATION

2.1 Definitions

Membrane volume (MV): 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.

2.2 Materials

Component	Material
HD Membrane	Porous polyacrylamide
Functional chemistry	Sulfonic Acid / t-Butyl
Housing	Polypropylene

2.3 Product Characteristics

	RECON HD-Sb
Nominal membrane volume (mL)	0.87
Membrane configuration	Flat sheet
Membrane bed thickness (mm)	0.55
Typical IgG binding capacity (mg/mL)	85 - 95
Flow rate range (mL/min)	4.4 - 13
Recommended flow rate (mL/min)	8.7
Flow rate range (MV/min)	5-15
Recommended flow rate (MV/min)	10
Maximum operating pressure (psi/bar)	90/6
Connections: Inlet/outlet	Female Luer
Vent	Female Luer

2.4 Chemical Compatibility

The compatibility of the Natrix Recon HD-Sb Membrane with a number of chemicals frequently used in biomolecule purification processes has been determined. Membrane samples were exposed to each chemical for 4 hours at room temperature. Subsequent to the chemical exposure, membrane performance was characterized by determining buffer flux through the membrane at 100 kPa applied pressure and human IgG dynamic binding capacity (measured at 10% breakthrough). Natrix Recon HD-Sb membranes are compatible with most buffers and solvents commonly used in chromatographic biomolecule purification processes, but have limited compatibility with Hypochlorite (1%).

This information should be used as a guide only, as chemical compatibility can be influenced by a number of conditions, including exposure time, temperature and chemical concentration.

CHEMICAL	SCORE
Acids	
1 M HCl	G
0.1 M HCl	E
Bases	
1 M NaOH	E
0.1 M NaOH	E
Alcohols	
Isopropanol	E
Methanol	E
70% Ethanol	E
50% Glycerol	E

CHEMICAL	SCORE
Ketones	
Acetone	E
Nitrogen-containing solutions	
Acetonitrile	E
6 M Guanidine	G
8 M Urea	E
Oxidative solutions	
2 wt % Hydrogen Peroxide	E
1% Hypochlorite	F
Surfactants	
1% SDS	G

E = Excellent, G = Good, F = Fair

SECTION 3: INSTALLATION AND SETUP

3.1 Storage Prior to Use

Natrix Recon HD-Sb devices should be stored in original packaging in a clean, dry location at room temperature and away from direct sunlight.

3.2 Connections

Natrix Recon HD-Sb devices are manufactured with female Luer connections on all ports. Adapters may be required to connect to the intended chromatography system which can be configured with M6 or 10-32 threaded connectors.

For example, the IDEX® P-656 connector will adapt the Natrix Recon HD-Sb connections to 10-32 threaded connections. (See www.idex-hs.com)

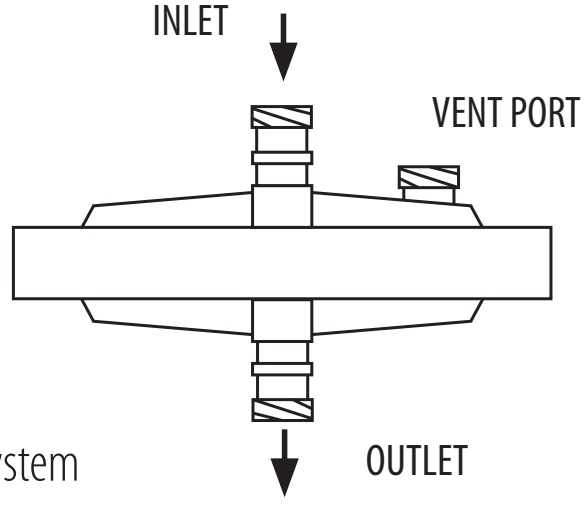
IDEX is a registered trademark of IDEX Corporation.

3.3 Installation

A visual inspection of the device before use is recommended to ensure that no damage has occurred during shipment. The Natrix Recon HD-Sb device is supplied with a blue ring to indicate the Sulfonic Acid/t-Butyl ligand chemistry.

Connecting the Recon Device

1. Natrix Recon HD-Sb devices have three ports: inlet, outlet, and vent. The inlet port is on the same side of the device as the vent port as shown in the figure at right.
2. Remove Luer caps from the inlet and outlet of the device.
3. Connect the inlet and outlet of the device to the chromatography system using the appropriate connector or adapter.



Priming

1. Open vent port. Start flowing equilibration buffer at 10 MV/min.

2. While flowing the the equilibration buffer, position the device at approximately 45 degrees such that the vent port is at the highest point to allow air removal.

3. Gently tap the device to facilitate air removal. Keep tapping until all the air bubbles have escaped through the vent port.

4. Replace the vent cap after all the air has been purged from the device.

5. With the flow on, temporarily turn the connected device with the outlet pointing up to remove any air trapped downstream from the membrane.

6. Once all the air bubbles have been removed, turn the device with the inlet pointing up.

Sanitizing

1. The recommended sanitization solution is 1 M NaOH.

2. Check if there are any air bubbles trapped in the device before sanitizing. Complete the priming procedure to remove any trapped air bubbles.

3. Flush the device with sanitization solution for 5 minutes at 10 MV/min, followed by a static soak for up to 60 minutes.

4. Flush the device with at least 100 mL of equilibration buffer at the desired flow rate or until pH and conductivity return to the specified range. A solution with up to 10x concentration of the equilibration buffer can be used to reduce the volume needed to achieve the desired pH prior to the equilibration step.

SECTION 4: OPERATION

4.1 Sample Preparation

The pH and conductivity of the sample should be appropriately adjusted before loading. The conductivity of the sample can be adjusted through dilution in most cases. For optimal binding capacity, the sample should be adjusted to pH 4.5 at 16 mS/cm or to pH 5.0 at 5 mS/cm.

Ensure the sample solution has enough buffering capacity at operating pH. Microfiltration of the process stream before loading is recommended to avoid excessive pressure increase during operation.

Binding conditions can be quickly screened using breakthrough experiments. The mAb breakthrough can be monitored by frontal analysis at A280 (in case of the Protein A elution pool having trace amounts of impurities) or using Protein A HPLC for flow through fractions (for a clarified harvest load).

For further guidance on the selection of buffering ions, pH and conductivity, refer to Section 5 of this document, and the NatriPur HD-Sb Method Development Guide at www.natrixseparations.com/guides.

4.2 Natrix Recon HD-Sb Process Steps

1. Equilibration

- Flow equilibration buffer at 10 MV/min flow rate for 5 to 10 minutes.
- Ensure effluent pH and conductivity are within the desired range.

2. Load

- Adjust pH and conductivity of sample and ensure the sample has enough buffering capacity at operating pH (approximately 20 mM).
- Microfilter and load the appropriate amount of sample solution (based on dynamic binding capacity testing).

3. Wash (Flush)

- Flow equilibration buffer at 10 MV/min for 4–5 minutes to complete the sample injection and wash out unbound impurities.
- If needed, flow a second wash buffer at 10 MV/min for 5-10 minutes to remove loosely bound impurities.*

**Some applications such as mAb capture chromatography may need a second wash step to remove loosely bound impurities. The conditions for this can be determined from linear gradient screening experiments. If initial fractions show a lower product purity than subsequent fractions during linear gradient elution, the second wash step may be useful in improving the product purity during step elution.*

4. Elution

- Flow elution buffer at 10 MV/min for 5 – 10 minutes to elute the product.

5. Cleaning/Regeneration

- Remove strongly bound impurities by flowing high salt buffer at neutral or slightly basic pH (for example, 500 mM NaCl in 50 mM sodium phosphate, pH 7.0 or 1 M NaCl, 25 mM Tris, pH 8.2) for 5 minutes at 10 MV/min flow rate.
- If the process demands a stronger cleaning agent, up to 1 M NaOH can be used.

6. Re-equilibration

- Flow equilibration buffer at 10 MV/min for 5 – 10 minutes.
- Ensure effluent pH and conductivity are within specified range.

4.4 Disconnecting & Disposal

Ensure that system pressure has been relieved prior to disconnecting the device.

Personal Protective Equipment should be worn when handling the device or during operation in accordance with any applicable safety protocols and standard operating procedures.

SECTION 5: PROCESS VARIABLES

Effect of Process Parameters

Optimization of process parameters such as pH, conductivity and flow rate is important to maximize the performance of any ion exchange chromatography media. The effect of these parameters on the performance such as binding capacity, impurity clearance and yield should be studied through screening experiments.

pH

Below its pI, a protein carries a net positive charge and binds to cation exchange media. The operational pH of the feed sample, as well as the wash and elution buffers, should be screened over a wide range of pH to maximize product binding, purity and yield. Typically, mAb binds in the pH range of 4.0 to 5.5. Mabs can be eluted by increasing the conductivity, or by increasing the pH by 1 to 2 units.

Buffers

Buffer species are important during ion-exchange chromatography to control the operating pH. Whenever possible, buffer ions should be anionic or zwitterionic, and the buffers and process feed streams should have sufficient buffering capacity to control the pH variations.

For example, for the purification of mAbs, the following conditions and buffers can be used:

Flow rate	8.7 mL/min
Equilibration/wash buffer	50 mM Acetate + NaCl, pH 4.5 & 16 mS/cm
Load	60 mg/mL
Elution	Linear gradient from A to B for 20 minutes
	Buffer A: 50 mM Acetate, pH 5.0
	Buffer B: 50 mM Acetate + 500 mM NaCl, pH 5.0
Cleaning/Regeneration	250 mM NaOH

Conductivity

The conductivity (or salt concentration) of the process feed stream affects the protein binding capacity of cation exchange media and should be screened to maximize product binding. The conductivity of the elution buffer should also be screened to maximize product purity and yield. Natrix HD-Sb has high mAb binding capacity over a wide conductivity range. Depending on pH, sample conductivity can be as high as 22 mS/cm.

Flow Rate

Natrix HD-Sb membrane has good binding capacity over a wide range of flow rates from 5 to 15 MV/min. The recommended flow rate is 10 MV/min which corresponds to 0.1 minute of residence time. The binding capacity changes ±15% when flow rate is changed to either 5 or 15 MV/min.

SECTION 6: TROUBLESHOOTING

PROBLEM	POTENTIAL CAUSE	ACTION
Leaking adsorber	Improper or loose connections	1. Verify correct connectors are installed properly 2. Ensure vent cap is installed
	Device integrity compromised	Replace device and verify maximum pressure was not exceeded
Air bubbles present	Incomplete air removal	Repeat priming procedure – see section 3.3
Incomplete product recovery	Insufficient buffer wash (flush)	Ensure post-loading buffer wash purges entire system fluid volume – see section 4.2
	Improper elution conditions	See recommended elution conditions in Section 5.

PROBLEM	POTENTIAL CAUSE	ACTION
Pressure exceeds operating limit or cannot achieve target flow rate	Debris or precipitate in process stream	Microfilter the process stream before loading
	Slow and continuous precipitation in process stream	Modify buffer conditions to promote stability
		Filter the process stream immediately before loading or use in-line filtration
	Chromatography system generates high back pressure	Modify equipment flow path (e.g. remove flow restrictor)
	Improper sanitization procedure	Replace device – use recommended sanitization procedure – see section 3.3
Poor impurity clearance	*For process troubleshooting and optimization, please refer to the Natrix HD-Sb Method Development Guide at www.natrixseparations.com or call your local distributor.*	

SECTION 7: ORDERING

For ordering information, please contact your local distributor. Distributor contact information can be found at www.natrixseparations.com/contact

Product Code	Product Name	Nominal Membrane Volume (mL)	Quantity /Pack
NSB-02	Natrix Recon HD-Sb	0.87	5

Contact Natrix Separations if larger HD-Sb membrane volumes are required to meet specific manufacturing needs.

SECTION 8: TECHNICAL SUPPORT

For technical support, please contact your local distributor. Distributor contact information can be found at: **www.natrixseparations.com/contact.**

MANUFACTURER'S WARRANTY

SELLER warrants for a period of twelve (12) months from date of delivery that the Products sold to BUYER will be free from defects in material or workmanship at time of delivery. SELLER's sole obligation for any nonconforming Products shall be to repair, or in its sole discretion, replace, any Products found by SELLER to have been defective at the time of delivery if (i) BUYER sets forth in writing to SELLER prior to the expiration of such 12-month period information describing the defective Product, including the type of Product, invoice number, shipment date, installation date and the product into which Product was installed, and a full description of any defect, sufficient for SELLER to determine if Product is defective and (ii) such Product is returned (at BUYER's expense and risk) and received by SELLER within fifteen (15) days after this warranty expires. Failure to comply with these requirements shall nullify and void this warranty. SELLER shall have a reasonable time to make repairs or replace a defective Product. All Product repaired, corrected, or replaced shall be subject to the same express warranties for the remainder of the original warranty period. SELLER reserves the right to utilize, as replacement parts, fully certified parts that have been re-manufactured.

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Seller shall notify the buyer of any changes to this warranty in compliance with the Seller's "Change Control" Standard Operating Procedure (SOP), in compliance with ISO 9001 quality standards.

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