

INSTRUCTION GUIDE

NatriFlo™ HD-Q Recon & Recon Mini
Flow-Through Membrane Adsorbers



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.

The membrane has been demonstrated to have a minimum usable life of 4 years and that of the adsorber is expected to be at least 5 years. The product is ready to use and should be stored under ambient conditions until required.

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

Read operating instructions carefully prior to use of NatriFlo membrane adsorbers.

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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. When packed into the NatriFlo HD-Q Membrane Adsorbers, this combination of performance and speed enables low risk, scalable polishing solutions for efficient purification of biologics.

NatriFlo adsorbers are easily scalable from the laboratory to GMP production and are designed to work with existing chromatography systems.

This instruction guide is only for NatriFlo HD-Q Recon & Recon Mini membrane adsorbers, 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 membrane adsorber. 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	Polyacrylamide composite
Functional chemistry	Quaternary amine
Housing	Polypropylene

2.3 Product Characteristics

	RECON MINI	RECON
Nominal membrane volume (mL)	0.2	0.8
Membrane configuration	Flat sheet	
Membrane bed thickness (mm)	0.5	
Minimum BSA binding capacity (mg) ¹	40	160
mAb polishing capacity (g) ²	2	8
Flow rate range (mL/min)	1 - 5	4 - 20
Flow rate range (MV/min)	5-25	
Maximum operating pressure (psi/bar)	75/5	90/6
Connections: Inlet/outlet	Female Luer	
Vent	n/a	Female Luer

1 10% breakthrough dynamic binding capacity at 10 MV/min in 25 mM Tris buffer, pH 8.1

2 Results will vary depending on sample characteristics

2.4 Chemical Compatibility

The compatibility of the Natrix HD-Q 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 water flux through the membrane at 100 kPa applied pressure and BSA dynamic binding capacity (measured at 10% breakthrough). Natrix HD-Q Membranes are compatible with most buffers and solvents commonly used in chromatographic biomolecule purification processes, but incompatible with Hypochlorite (1%) and SDS (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	E
0.1 M HCl	E
Bases	
1 M NaOH	F
0.1 M NaOH	G
1 M NaOH + 2 M NaCl	E
0.5 M NaOH + 2 M NaCl	E
Alcohols	
Isopropanol	E
Methanol	G
70% Ethanol	E
50% Glycerol	E

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

E = Excellent, G = Good, F = Fair, NR = Not recommended

2.5 Buffer Selection

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

As a starting point, the following buffers can be used:

- Equilibration Buffer: 25 mM Tris-HCl pH 8.1
- Stripping / Cleaning Buffer: 25 mM Tris-HCl + 1 M NaCl pH 8.1
- Sanitization Solution: 1 M NaOH containing 2 M NaCl

Please refer to NatriFlo HD-Q Method Development Guide at www.natrixseparations.com for further guidance on buffer selection.

SECTION 3: INSTALLATION AND SETUP

3.1 Storage Prior to Use

NatriFlo HD-Q membrane adsorbers should be stored in original packaging in a clean, dry location at room temperature and away from direct sunlight.

Real-time and accelerated shelf-life studies are ongoing. The membrane has been demonstrated to have a minimum usable life of 4 years, and that of the adsorber unit is expected to be at least 5 years.

3.2 Connections

NatriFlo HD-Q Recon and Recon Mini membrane adsorbers 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.

An example is the IDEX® P-656 connector which will adapt NatriFlo Recon and Recon Mini connections to 10-32 threaded connections. (See <http://webstore.idex-hs.com/>)

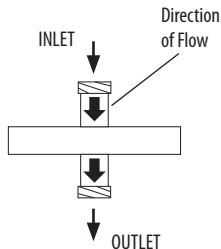
IDEX is a registered trademark of IDEX Corporation.

3.3 Installation

A visual inspection of the adsorber before use is recommended to ensure that no damage has occurred during shipment. The NatriFlo HD-Q Recon and Recon Mini are supplied with an orange ring to indicate the quaternary amine membrane chemistry.

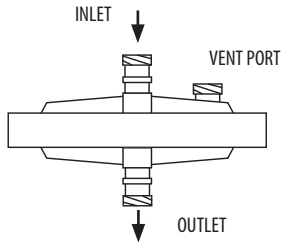
Connecting the Recon Mini Adsorber

1. NatriFlo HD-Q Recon Mini adsorbers have only two ports: inlet and outlet. The fluid flow direction is indicated by an arrow molded into each port as shown in the figure at right.
2. Remove Luer caps from the inlet and outlet of the adsorber.
3. Connect the inlet and outlet of the adsorber to the chromatography system using the appropriate connector or adapter.
4. **For HD-Q Recon Mini adsorbers only:** a system backpressure of at least 20 psi is required for optimum performance.



Connecting the Recon Adsorber

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



Priming

1. Flow equilibration buffer at 10 MV/min for approximately 5 minutes.
2. Orient the adsorber with the outlet facing up to maximize air removal.
3. If air bubbles are visible coming through the outlet of the adsorber, gently tap or shake to dislodge trapped air.
4. **For Recon adsorbers only:** If air bubbles are visible in the inlet chamber of Recon adsorbers, tilt the adsorber so that the vent port is at the highest point, remove the vent cap, and tap gently to remove any trapped air through the open vent port. Replace the vent cap after any air has been purged.
5. Once priming is complete, the adsorber can be operated in any orientation.

Sanitizing (optional)

1. The recommended sanitizing solution is 1 M NaOH containing 2 M NaCl.
2. To sanitize the adsorber, first complete the priming procedure with equilibration buffer.
3. Flush the adsorber with sanitizing solution for 5 minutes at 10 MV/min, followed by a static soak for up to 60 minutes.
4. Flush the adsorber with at least 50 MV of equilibration buffer at the desired flow rate or until pH and conductivity return to the specified range. The use of a higher concentration buffer (e.g. 10X equilibration buffer concentration) can reduce the buffer flush volume required after sanitization.

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. However, diafiltration may be necessary in some cases to remove specific interfering ions. For example, the presence of citrate ions in the process stream reduces the performance of any anion exchange media and may require an ultrafiltration/diafiltration step before anion exchange chromatography.

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.

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

4.2 NatriFlo HD-Q Process Steps

1. Equilibration

- Flush adsorber with approximately 50 MV of equilibration buffer.
- Ensure effluent pH and conductivity are within the desired range.

2. Load

- Load sample at desired flow rate to intended adsorber capacity.
- Collect flow-through fractions as required for analysis.

3. Wash (Flush)

- Use 10-40 MV of equilibration buffer (or as required) to flush adsorber and complete product recovery.

4. Strip

- Strip using 1-2 M NaCl in equilibration buffer if bound impurities need to be eluted (e.g. to understand mass balance and characteristics of impurities).

4.3 Disconnection and Disposal

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

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

SECTION 5: PROCESS VARIABLES

pH

The pH of the solution should be optimized so the target species are negatively charged during loading. For polishing of monoclonal antibodies, buffer pH is typically between 6 and 8.5.

Conductivity

NatriFlo HD-Q membrane adsorbers have demonstrated very good DNA, HCP, endotoxin and viral clearance for high loads at conductivities up to 15 mS/cm.

Flow Rate

NatriFlo HD-Q membrane adsorbers maintain good binding capacity over a wide range of flow rates up to 25 MV/min.

Protein Concentration

NatriFlo HD-Q membrane adsorbers have shown excellent HCP reduction for loads over a wide range of mAb concentrations up to 10 kg mAb/L of membrane.

Buffers

For superior performance, buffers used with NatriFlo HD-Q membrane adsorbers should be cationic or zwitterionic and have sufficient buffering capacity at operating pH.

For further guidance on process development, refer to NatriFlo HD-Q Method Development Guide at www.natrixseparations.com.

SECTION 6: TROUBLESHOOTING

PROBLEM	POTENTIAL CAUSE	ACTION
Leaking adsorber	Improper or loose connections	<ol style="list-style-type: none">1. Verify correct connectors are installed properly2. Ensure vent cap is installed (Recon only)3. Replace faulty connectors
	Adsorber integrity compromised	Replace adsorber 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

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 adsorber – use recommended sanitization procedure – see section 3.3
Poor impurity clearance	<i>For process troubleshooting and optimization, please refer to the NatriFlo HD-Q Method Development Guide at www.natrixseparations.com or call your local distributor.</i>	

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
NXF-01	NatriFlo HD-Q Recon Mini	0.2	10
NXF-02	NatriFlo HD-Q Recon	0.8	5
NXF-10	NatriFlo HD-Q Pilot	15	1
NXF-20	NatriFlo HD-Q Process 150	115	1
NXF-50	NatriFlo HD-Q Process 600	460	1

Contact Natrix Separations if larger HD-Q 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. Additional technical resources, including the documents listed below, are available on the Natrix Separations website.

Please visit: **www.natrixseparations.com/guides**

- NatriFlo HD-Q Recon and Recon Mini Quick Start Guide
- NatriFlo HD-Q Methods Development Guide
- NatriFlo HD-Q Product Selection Guide
- NatriFlo HD-Q Data File

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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