

INSTRUCTION GUIDE

NatriFlo™ HD-Q

Pilot & Process

Flow-Through Membrane Adsorbers



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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. Manufacturing of Pilot and Process units is carried out in a cleanroom facility that meets or exceeds FDA Good Manufacturing Practice standards through voluntary compliance.

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



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 Pilot and Process 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 hydrogel reinforced with polypropylene mesh
Functional chemistry	Quaternary amine
Internal core/guard	Polypropylene
Inlet/Outlet flange	Polypropylene
Capsule Housing	Polypropylene

2.3 Product Characteristics

	Pilot	Process 150	Process 600	
Nominal membrane volume (mL)	15	115	460	
Membrane configuration	Pleated sheet			
Membrane bed thickness (mm)	0.5			
Minimum BSA binding capacity (g) ¹	3	23	92	
mAb polishing capacity (g) ²	150	1150	4600	
Flow rate range (mL/min)	75 - 375	600 - 3000	2300-11500	
Flow rate range (MV/min)	5 - 25			
Maximum operating pressure (psi/ bar)	75/5	90/6	90/6	
Connections:	Inlet/outlet	3/4" Sanitary (TC)	1" Sanitary (TC)	1" Sanitary (TC)
	Vent	Luer with Cap	Sanitary Vent	Sanitary Vent
	Drain	Luer with Cap	1/4" Sanitary Drain	1/4" Sanitary Drain

1 10% breakthrough dynamic binding capacity of Bovine Serum Albumin 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

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.

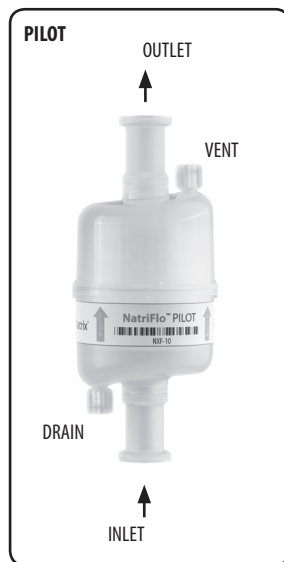
3.2 Connecting and Priming the PILOT Adsorber

A visual inspection of the adsorber before use is recommended to ensure that no damage has occurred during shipment.

- Process flow direction is indicated by an arrow on the housing and the product label.
- Install the adsorber in an upright position with inlet at the bottom and the outlet at the top.
- Connect the outlet and inlet of the adsorber to the skid or pump with sanitary connectors and gaskets.
- Open the vent on the outlet side of the adsorber.
- Flush the adsorber with equilibration buffer at 10 MV/min for approximately 5 minutes or until pH and conductivity signals are stable.
- Close the vent when liquid is coming out.

Note: operator may have to tilt the adsorber to remove the air in the inlet and outlet.

- Continue filling until no air can be seen coming out of the outlet.
- In case of visible air bubbles, gently tap or shake the adsorber to dislodge trapped air.



3.3 Connecting and Priming the PROCESS Adsorbers

A visual inspection of the adsorber before use is recommended to ensure that no damage has occurred during shipment.

- Process flow direction is indicated by an arrow on the housing and the product label.
- Connect the outlet and inlet of the adsorber to the skid or pump with sanitary connectors and gaskets while keeping the adsorber in the upright position.
- Open the vent on the top of the adsorber.

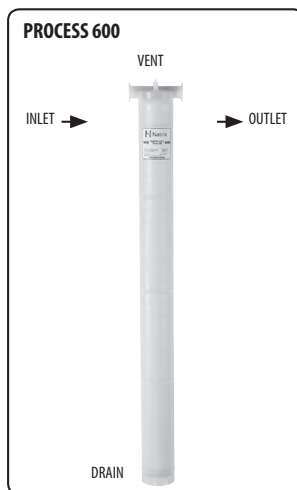
d. Flush the adsorber with equilibration buffer at 10 MV/min for approximately 5 minutes or until pH and conductivity signals are stable.

e. Close the vent when liquid is coming out.

Note: operator may have to tilt the adsorber to remove the air in the inlet and outlet.

f. Continue filling until no air can be seen coming out of the outlet.

g. If air bubbles are visible coming through the outlet of the adsorber, gently tap or shake to dislodge trapped air.



3.4 Sanitizing (optional)

- The recommended sanitizing solution is 1 M NaOH containing 2 M NaCl.
- To sanitize the adsorber, first complete the priming procedure with equilibration buffer.
- Flush the adsorber with sanitizing solution for 5 minutes at 10 MV/min, followed by a static soak for up to 60 minutes.
- Flush the adsorber with equilibration buffer at the desired flow rate until pH and conductivity return to the specified range (use at least 100 MV). The use of a higher concentration buffer (e.g. 10 times equilibration buffer concentration) can reduce the buffer flush volume required after sanitization.

SECTION 4: OPERATION

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

4.1 Sample Preparation

The pH and conductivity of the sample should be appropriately adjusted before loading. To ensure proper scaling performance, ensure the conditions of the sample solution and the process parameters are maintained similar to the protocol developed at laboratory scale.

4.2 Scale-Up

Scaling up a process within the NatriFlo product line is accomplished using calculations based on the membrane volume. When scaling up, the parameters that change linearly with membrane volume are the binding capacity and the process flow rate. The scale-up factors listed in the table below are the multipliers for both binding capacity and flow rate when scaling up from a process developed at the Recon Mini scale.

PRODUCT	MV (mL)	Scale-Up Factor
Recon Mini	0.2	1
Recon	0.8	4
Pilot	15	75
Process 150	115	575
Process 600	460	2300

The scale-up factor indicates the expected increase in binding capacity between adsorber sizes. If the desired increase in binding capacity is between adsorber products, select the next larger product from the table. To maintain consistent performance when scaling up, use the scale-up factor to determine the flow rate for the larger adsorber.

Pipe and system volumes in different-sized chromatography systems can vary significantly, and should be considered when determining fluid volumes. Minimizing tubing size and length in chromatography systems can reduce buffer consumption during adsorber operation.

4.3 NatriFlo HD-Q Processing Steps

1. Sanitization and Pre-Equilibration

- If required, sanitize with 1M NaOH containing 2M NaCl (see section 3.4).

2. Equilibration

- Flush adsorber with approximately 50 MV of equilibration buffer.
- This step can be omitted if the adsorber has been sanitized and flushed with 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).

4.4 Disconnection and Disposal

Ensure that all system pressure has been relieved prior to disconnecting the adsorber. NatriFlo adsorbers are equipped with a drain port at the bottom to drain all fluids if required.

NatriFlo HD-Q Pilot and Process adsorbers are intended to be used for processing of a single batch, and should be disposed of after completion of the unit operation for that batch.

SECTION 5: STRUCTURAL INTEGRITY TEST

This section describes the method for testing Natrix membrane adsorbers for structural integrity using a pressure / diffusion test.

5.1 Overview

The device is first primed with buffer to thoroughly hydrate the membrane and then drained. Compressed air is then used to apply a constant pressure through the device via the inlet and the flow rate is measured. The result provides an indication of whether or not there are any gross defects in the membrane and/or the housing. This method is suitable for products **NXF-10, NXF-20 and NXF-50**.

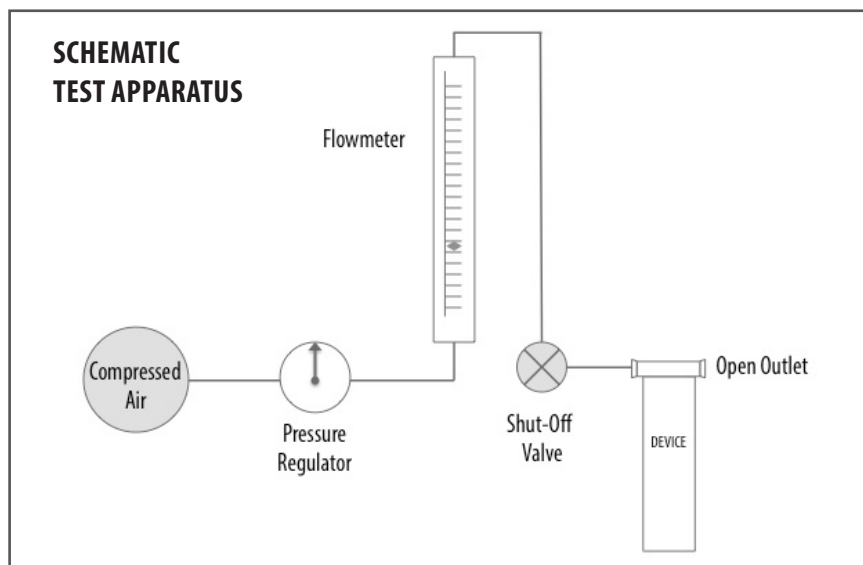
5.2 Method

1. As described in Section 3, install and prime the device by filling with the equilibration buffer of choice at ambient temperature.
2. Allow to soak for 5 minutes and then drain.
 - a. Open the drain plug and allow fluid to flow out of both the inlet and the outlet. Drain thoroughly.
3. The figure on page 13 shows a schematic representation of the test apparatus.
 - a. Prior to testing the device, check that the test system is air-tight and sealed to avoid false results. With the outlet capped, there should be no flow under pressure.
 - b. The shut-off valve is used to protect the flow meter from liquid that may be drawn back through the system. The valve must be closed before connecting the device and before decreasing the pressure at the end of the test.
 - c. Automated test equipment that can process the required test parameters can also be used.
4. With the apparatus assembled and the device connected, open the compressed air source.
 - a. Set the regulator to apply a pressure of 2.0 ± 0.1 psi.
5. Allow the system to stabilize for 3 minutes and then record the flow rate.

6. The maximum flow rate for any membrane adsorber under 2.0 psi pressure should not exceed 100 ml/min.
 - a. This figure represents an upper limit for diffusive flow across the membrane.
 - b. Above this limit, bulk flow may be occurring, which is indicative of a possible defect in the membrane and/or device housing that could cause immediate bypass and loss of function.
 - c. The table lists other factors that could cause test failure, which should be checked and ruled out before concluding that the product is defective.

5.3 Reasons for Test Failure (other than device defect)

CAUSE	REMEDY
Membrane incorrectly hydrated	Use correct priming medium (equilibration buffer)
Membrane not sufficiently hydrated	5 minutes soak required
System not sealed	Check all fittings, joints etc and ensure vents are shut
System not sufficiently stabilized	3 minutes stabilization required
Incorrect test pressure used	2.0 ± 0.1 psi required
Different test gas used	Method has been developed for air
Test not performed at suitable temperature	Method has been developed for ambient conditions ($22 \pm 2^\circ\text{C}$)



SECTION 6: TROUBLESHOOTING

▲ PROBLEM: **Leaking Adsorber**

POTENTIAL CAUSE	ACTION
Improper or loose connections	<ol style="list-style-type: none"> 1. Verify correct connectors are installed properly 2. Ensure vent cap and drain caps are installed 3. Replace faulty connectors
Adsorber integrity compromised	Replace adsorber and verify maximum pressure was not exceeded

▲ PROBLEM: **Air bubbles present**

POTENTIAL CAUSE	ACTION
Incomplete air removal	Repeat priming procedure – see section 3.2/3.3

▲ PROBLEM: **Incomplete product recovery**

POTENTIAL CAUSE	ACTION
Insufficient buffer wash (flush)	Ensure post-loading buffer wash purges entire system fluid volume – see section 4.3

▲ PROBLEM: **Maximum pressure exceeded or cannot achieve target flow rate**

POTENTIAL CAUSE	ACTION
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
Adsorber integrity compromised	Replace adsorber and verify maximum pressure was not exceeded

▲ PROBLEM: **Poor impurity clearance**

For process troubleshooting and optimization, please refer to the NatriFlo HD-Q 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 per 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

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**](http://www.natrixseparations.com/guides)

- 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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