ANX **Sepharose** 4 Fast Flow (high sub)

Ion exchange resin

Instructions for Use

ANX Sepharose™ 4 Fast Flow (high sub) ion exchanger is a weak ion exchanger based on cross-linked agarose. It can be used with high flow rates and is well suited for process-scale chromatography. ANX Sepharose 4 Fast Flow (high sub) is part of the range of separation resins called BioProcess™ resins.

BioProcess chromatography resins are developed and supported for process-scale chromatography. BioProcess resins are produced with validated methods and are tested to meet manufacturing requirements. Secure ordering and delivery routines give a reliable supply of resins for production-scale. Regulatory Support Files (RSF) are available to assist process validation and submission to regulatory authorities. BioProcess resins cover all purification steps from capture to polishing.

Table of Contents

1 Characteristics 3
2 Column packing 6
3 Evaluating the packing 9
4 Maintenance 12
5 Process optimization 14
6 Troubleshooting guide 15
7 Ordering information 17

Read these instructions carefully before using the products.

Safety

For use and handling of the products in a safe way, refer to the Safety Data Sheets.

1 Characteristics

ANX Sepharose 4 Fast Flow (high sub) is based on cross-linked agarose, which gives the resin high chemical and physical stabilities. Additionally, the coupling chemistry enables the resin to withstand the harsh conditions encountered during sanitization and cleaning-in-place. In comparison with other ion exchangers based on Sepharose Fast Flow, the amount of agarose in the base matrix is lower. This results in a resin with high porosity, making ANX Sepharose 4 Fast Flow (high sub) particularly useful for the purification of high molecular-mass proteins or when different selectivity is needed.

ANX Sepharose 4 Fast Flow (high sub) is well-suited for process-scale chromatography and can be run at high flow rates, even when bed heights are relatively high, approximately 20 cm (see *Figure 1, on page 4*).

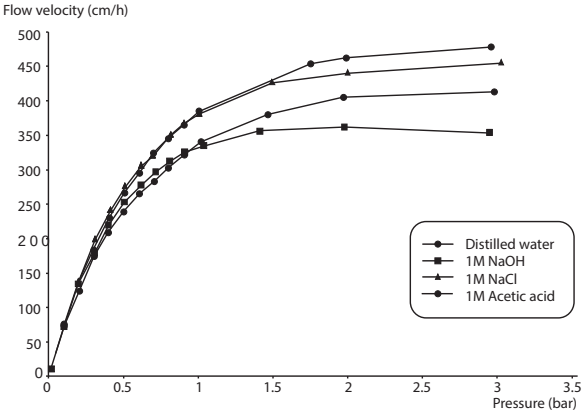


Fig 1. Pressure/flow curves for ANX Sepharose 4 Fast Flow (high sub) in BPG 100/500 column (i.d. 10 cm, bed height 20 cm) using different eluents

ANX Sepharose 4 Fast Flow (high sub) is a weak anion exchanger. The ion exchange group is a diethylaminopropyl group, see below.

Table 1. Characteristics of ANX Sepharose 4 Fast Flow (high sub)

Matrix	Cross-linked agarose, 4%, spherical
Particle size, d_{50v} [1]	~ 90 µm
Ion exchange type	Weak anion
Ionic capacity	0.13 to 0.18 mmol Cl^-/mL resin
Exclusion limit [M_r] globular proteins [2]	3×10^7
Pressure/flow characteristics	≥ 200 cm/h at 0.1 MPa in a XK 50/60 column with 5 cm diameter and 25 cm bed height (at 20 using buffers with the same viscosity as water)[3]
Operating temperature	4°C to 30°C

pH stability, operational [4]	3 to 13
pH stability, CIP [5]	2 to 14
pH ligand fully charged [6]	Below 9
Chemical Stability	Stable to commonly used aqueous buffers, 1.0 M NaOH[7] 8 M urea 8 M guanidine hydrochloride 70% ethanol 1 M acetic acid
Storage	20% ethanol, 4°C to 30°C

1 Median particle size of the cumulative volume distribution

2 Exclusion limit measured on the base matrix

3 The pressure/flow characteristics describes the relationship between pressure and flow under the set circumstances. The pressure given shall not be taken as the maximum pressure of the resin.

4 pH range where resin can be operated without significant change in function

5 pH range where resin can be subjected to cleaning- or sanitization-in-place without significant change in function

6 pH range where ligand is fully charged; although the ligand is fully charged throughout the range stated, only use the resin within the stated stability ranges.

7 1.0 M NaOH should only be used for cleaning purposes.

The titration curve in *Figure 2, on page 6* shows the pH working range of ANX Sepharose 4 Fast Flow (high sub), that is, the pH range over which the group is charged.

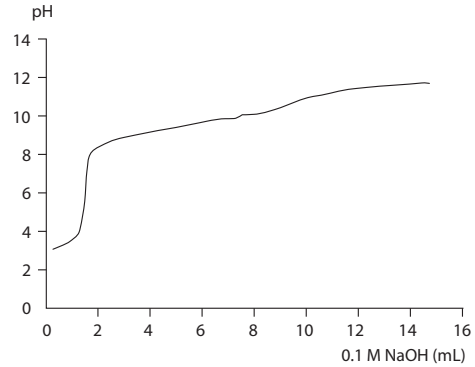


Fig 2. Titration curve of ANX Sepharose 4 Fast Flow (high sub)

2 Column packing

ANX Sepharose 4 Fast Flow (high sub) is supplied in suspension in 20% ethanol. Decant the 20% ethanol solution and replace with starting buffer before use.

Recommended columns

The columns from Cytiva listed below are recommended for use with ANX Sepharose 4 Fast Flow (high sub).

- XK columns

  Inner diameters of 16 mm, 26 mm and 50 mm; bed volumes up to 295 mL at bed heights of 15 cm

- Tricorn™ columns

  Inner diameters of 5 mm and 10 mm; bed volumes up to 24 mL at bed heights of max 30 cm

- HiScale™ columns

  Inner diameter of 16 mm, 26 mm and 50 mm; bed volumes up to 785 mL at bed heights of max 40 cm

Pilot and production-scale columns

- AxiChrom™ columns

  Inner diameters from 50 to 200 mm; bed volumes up to 16.7 L at bed heights of max 50 cm

- AxiChrom columns

  Inner diameters from 300 to 1600 mm; bed volumes up to 1005 L at bed heights of max 50 cm

- BPG variable bed, glass columns

  Inner diameters from 100 to 450 mm; bed volumes from 2.4 to 43 L, bed height max 30 cm (27 cm for BPG 450)

- Chromaflow™ variable and fixed bed columns

  Inner diameters from 180 mm

Packing large-scale columns

General recommendations

The packing method used depends on the type of chromatography resin, the type of column, and the equipment in use. Always read and follow the relevant column instruction manual carefully.

Sepharose Fast Flow ion exchangers are easy to pack since their rigidity allows the use of high flow rates, see *Figure 1, on page 4*. Typical bed height range from 10 to 15 cm. The different packing methods that are suitable for ANX Sepharose 4 Fast Flow (high sub) are listed below.

- Pressure packing (for columns with adaptors), for example, BPG columns
- Suction packing (for large columns with fixed bed heights)
- Hydraulic axial compression packing, for example, AxiChrom columns
- Chromaflow packing method for standard Chromaflow columns

How well the column is packed will have a major effect on the result of the separation. It is therefore very important to pack and test the column according to the following recommendations. Always begin by determining the optimal packing flow rate. Guidelines for determining optimal packing flow rates for columns with adaptors and fixed bed heights are given in the next section.

Determining optimal packing flow rates

The optimal packing flow rate is dependent on temperature, column size and type, resin, and volume. As a result, the optimal packing flow rate must be determined empirically for each individual system. To determine the optimal packing flow rate, proceed as follows:

Step	Action
1	Calculate the exact amount of resin needed for the slurry (this is especially important for columns with fixed bed heights). The quantity of resin required per liter packed volume is approximately 1.15 L sedimented resin.
2	Set up the column as for packing according to the instructions in the column manual.

Step	Action
3	Begin packing the resin at a low flow rate (e.g., 30% of the max flow rate, see *Figure 1, on page 4*).
4	Increase the pressure in increments and record the flow rate when the pressure has stabilized. Do not exceed the maximum pressure of the column.
5	The maximum flow rate is reached when the pressure/flow curve levels off or the maximum pressure of the column is reached. Stop the packing and do not exceed this flow rate. The optimal pressure/packing flow rate is 70% to 100% of the maximum pressure/flow rate, see *Figure 1, on page 4*.
6	Plot the pressure/flow rate curve as in *Figure 1, on page 4* and determine the optimal packing flow rate. The operational flow rate/ pressure must be < 70% of the packing flow rate/pressure.

Packing your column

Follow the detailed instructions in the column user manual supplied with your column. Copies can be ordered from your local Cytiva representative.

3 Evaluating the packing

To check the quality of the packing and to monitor this during the working life of the column, column efficiency must be tested directly after packing, at regular intervals afterwards, and when separation performance is seen to deteriorate. The best method of expressing the efficiency of a packed column

is in terms of height equivalent to a theoretical plate, HETP, and the peak asymmetry factor, A_s. These values are easily determined by applying a sample such as 1% acetone solution to the column. (Avoid coloured compounds since they can interact with the resin.)

It is important to realize that the calculated plate number will vary depending on the test conditions and it must therefore be used as a reference value only. It is also important that conditions and equipment are kept constant so that results are comparable. Changes in solute, solvent, eluent, sample volume, flow rate, liquid pathway, temperature, etc., will influence the results. For optimal results, the sample volume must be 1% to 3% of the column volume, and the flow velocity 15 to 30 cm/h.

If an acceptance limit is defined in relation to column performance, the column plate number can be used as part of the acceptance criteria for column use.

Method for measuring HETP and A_s

To avoid dilution of the sample, apply it as close to the column inlet as possible.

Conditions	
Sample volume	1.0% to 3.0% of bed volume
Sample concentration	1.0% (v/v) acetone in water, 0.8 M NaCl
Eluent	Water, 0.5 M NaCl in water or dilute buffer
Flow	20 to 30 cm/h
Detection	
Acetone	UV 280 nm
NaCl, buffer	Conductivity

Calculate HETP and A_s from the UV curve (or conductivity curve) as follows:

$$HETP = L/N$$

and $N = 5.54(V_R/W_h)^2$

where V_R = Retention volume

W_h = Peak width at half peak height

L = Bed height

N = Number of theoretical plates

V_R and W_h are in the same units.

To facilitate comparison of column performance the concept of reduced plate height is often used. The reduced plate height is calculated thus:

$$HETP / d$$

where d is the mean diameter of the particles. As a guide, a value of < 3 is normally acceptable.

The peak must be symmetrical, and the asymmetry factor as close as possible to 1 (values between 0.8 and 1.5 are usually acceptable). A change in the shape of the peak is usually the first indication of bed deterioration due to use.

Peak asymmetry factor calculation

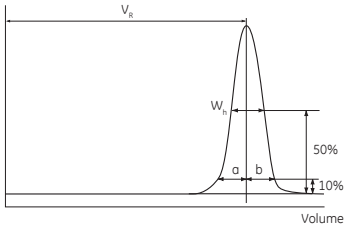
$$A_S = b/a$$

where

a = 1st half peak width at 10% of peak height

b = 2nd half peak width at 10% of peak height

The figure below shows a UV trace for acetone in a typical test chromatogram in which the HETP and As values are calculated.



Column: BPG 300
Resin: Sepharose Fast Flow
Bed height: 57.5 cm
Bed volume: 40.6 L
Sample: 1.05 L (1% acetone)
Eluent: Distilled water
Flow velocity: 19 cm/h
Wh = 0.9
HETP = 0.024 cm
a: 0.90
b: 0.85
A_S: 0.94

Fig 3. UV trace for acetone in a typical test chromatogram showing the HETP and A_S value calculations

4 Maintenance

For best performance of ANX Sepharose 4 Fast Flow (high sub) over a long working life, follow the procedures described below.

Equilibration

After packing, and before a chromatographic run, equilibrate with working buffer by washing with at least 5 bed volumes.

Regeneration

After each separation, elute any reversibly bound material either with a high ionic strength solution (e.g., 1 M NaCl in buffer) or by increasing pH. Regenerate the resin by washing with at least 5 bed volumes of buffer, or until the column effluent shows stable conductivity and pH values.

Cleaning-in-place (CIP)

Cleaning-in-place (CIP) is a cleaning procedure that removes contaminants such as lipids, precipitates, or denatured proteins that can remain in the packed column after regeneration. Such contamination is especially likely when working with crude materials. Regular CIP also prevents the buildup of these contaminants in the resin bed and helps to maintain the capacity, flow properties, and general performance of the resin.

A specific CIP protocol must be designed for each process according to the type of contaminants present. The frequency of CIP depends of the nature and the condition of the starting material, but one CIP cycle is generally recommended every 1 to 5 separation cycles.

Standard CIP protocol:

Step	Action
1	Wash with 0.5 column volumes of filtered 2 M NaCl. Contact time 10 to 15 min. Reversed flow direction.
2	Wash with 1.0 M NaOH at 40 cm/h. Contact time 1 to 2 hours. Reverse flow direction. Wash with 2 to 4 column volumes of 0.55 nonionic detergent in 1 M acetic acid. Contact time 1 to 2 hours. Reversed flow direction. Alternatively, wash with 2 to 4 column volumes of up to 70% ethanol or 30% isopropanol. Contact time 1 to 2 hours. Reversed flow direction.

CAUTION
Specific regulations may apply when using 70% ethanol or 30% isopropanol since it can require the use of explosion-proof areas and equipment.

Sanitization

To reduce microbial contamination in the packed column, sanitization using 0.5 to 1.0 M NaOH with a contact time of 1 hour is recommended.

Sterilization

Autoclaving is the only recommended sterilization treatment. Equilibrate the resin with 0.5 M NaCl, pH 7. Dismantle the column and autoclave the resin at 120°C for 30 minutes. Sterilize the column parts according to the instructions in the column manual. Re-assemble the column, then pack and test it as recommended.

Storage

Unused resin can be stored in the container at 4°C to 30°C. Make sure that the screw-top is fully tightened. Packed columns must be equilibrated in working buffer containing 20% ethanol to prevent microbial growth.

5 Process optimization

For further information and details about process optimization and scale-up, consult the following handbooks produced by Cytiva:

Ion Exchange Chromatography: Principles and Methods, 11000421

Handbook of Process Chromatography, 18112156

6 Troubleshooting guide

High back pressure

Step	Action
1	Check that all valves between the pump and the collection vessel are fully open.
2	Check that all valves are clean and free from blockage.
3	Check if equipment in use up to and after the column is generating any back pressure. (For example, valves and flow cells of incorrect dimensions.)
4	Perform CIP to remove tightly bound material from the resin.
5	Check column parts, such as filters and bed supports, according to the column instruction manual.

Unexpected chromatographic results

Step	Action
1	Check the recorder speed/signal.
2	Check the flow rate.
3	Check the buffers.

Step	Action
4	Check that there are no gaps between the adapter and the resin bed, or back mixing of the sample before application.
5	Check the efficiency of the column packing, see *Chapter 3 Evaluating the packing, on page 9*.
6	Check if there have been any changes in the pretreatment of the sample.

Infections

Step	Action
1	Check the connections and prefilters.
2	Check the components, for example, the buffers and sample components.
3	Check that the column has been properly sanitized.

Trapped air

Step	Action
1	Check that the buffers are equilibrated to the same temperature as the packed column.
2	Check that there are no loose connections or leaking valves.

If air has entered the column, the column must be repacked. However, if only a small amount of air has been trapped on top of the bed, or between the adapter net and head, it can be removed by pumping eluent in the opposite direction. After this, check the efficiency of the packed bed (see *Chapter 3 Evaluating the packing, on page 9*) and compare the result with the original efficiency values.

7 Ordering information

Product	Quantity	Product code
ANX Sepharose 4 Fast Flow (high sub)	25 mL	17128710
	500 mL	17128701
	5 L	17128704
	10 L	17128705
	60 L[1]	17128760

[1] Pack size available upon request

Supplied in suspension in 20% ethanol

Handbook	Product code
Ion Exchange Chromatography: Principles and Methods	11000421
Handbook of Process Chromatography	18112156

For information about process scale columns, ask for the following Data files:

AxiChrom	28929041
BPG columns	18111523
Chromaflow columns	18111884

For additional information, including Application Notes, References and Regulatory Support Files, contact your local Cytiva representative.

Page intentionally left blank

cytiva

cytiva.com

For local office contact information, visit cytiva.com/contact

71501728 AE V:7 07/2020