

ANX Sepharose™ 4 Fast Flow (high sub)

Ion exchange chromatography resin

Instructions for Use

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

ANX Sepharose 4 Fast Flow (high sub) provides:

- high binding capacity and good flow properties
- high chemical and physical stability
- straightforward and effective cleaning-in-place (CIP) and sanitization
- higher porosity suitable for purifying proteins with a high molecular weight

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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 Introduction

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.

2 Resin properties

ANX Sepharose 4 Fast Flow (high sub) is based on cross-linked agarose, which gives the resin a high chemical and physical stability. The coupling chemistry enables the resin to withstand the harsh conditions during sanitization and CIP. 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 [Fig. 1, on page 4](#)).

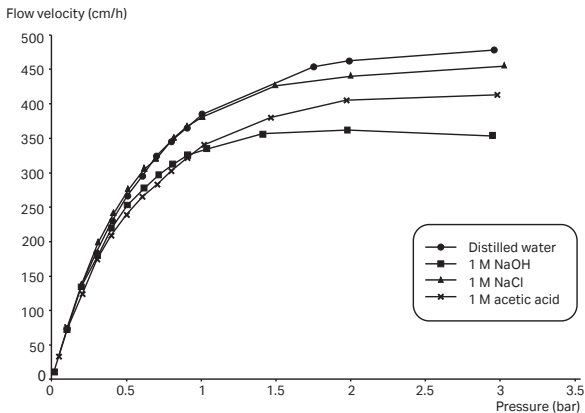


Fig 1. Pressure-flow curves for ANX Sepharose 4 Fast Flow (high sub) in BPG 100/500 column (i.d. 10 cm, bed height 20 cm) using different eluents.

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

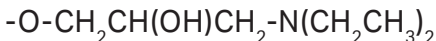


Fig 2. Partial structure of ANX Sepharose 4 Fast Flow (high sub).

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

Matrix	Cross-linked agarose, 4%, spherical
Particle size, d_{50v}¹	~ 90 μ m
Ion exchange type	Weak anion
Ionic capacity	0.13 to 0.18 mmol Cl ⁻ /mL resin
Exclusion limit [M_r] globular proteins²	~ 3×10^7
Pressure-flow characteristics³	≥ 200 cm/h at 0.1 MPa in a XK 50/60 column with 5 cm diameter and 25 cm bed height (at 20°C using buffers with the same viscosity as water)
Operating temperature	4°C to 30°C
pH stability, operational⁴	3 to 13
pH stability, CIP⁵	2 to 14
pH ligand fully charged⁶	Below 9
Chemical stability	Stable in commonly used aqueous buffers: • 1.0 M NaOH (only to be used for cleaning purposes) • 8 M urea • 8 M guanidine HCl • 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 describe 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.

The titration curve in [Fig. 3, 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.

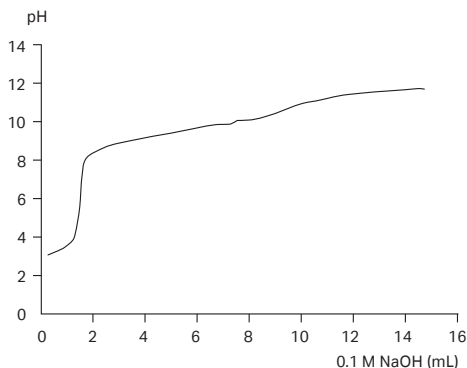


Fig 3. Titration curve of ANX Sepharose 4 Fast Flow (high sub).

3 Column packing

Introduction

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

Laboratory-scale columns

Column	Inner diameter (mm)	Bed volume (mL)	Bed height (cm)
XK columns	16, 26, 50	max. 295	15
Tricorn™ columns	5, 10	max. 24	max. 30
HiScale™ columns	10, 16, 26, 50	max. 785	max. 40

Pilot- and production-scale columns

Column	Inner diameter (mm)	Bed volume (L)	Bed height (cm)
AxiChrom™ columns	50–200	max. 16.7	max. 50
	300–1600	max. 1005	max. 50
BPG variable bed, glass columns	100–450	max. 2.4–43	max. 30*
Chromaflow™, variable and fixed bed columns	400–†	†	†

* Max. 27 cm for BPG 450.

† For more details, refer to Data file CY13377.

Packing large-scale columns

General recommendations

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

Sepharose Fast Flow ion exchangers are easy to pack since their rigidity allows the use of high flow values, see [Fig. 1, on page 4](#). A typical bed height ranges 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 adapters), for example, BPG columns

- suction packing (for large columns with fixed bed heights)
- hydraulic axial compression packing, for example, AxiChrom columns
- Chromaflow packing for standard Chromaflow columns

The packing quality of the column greatly affects the separation results. It is very important to pack and test the column according to the following recommendations. Water, ethanol solution, or buffer solution are suitable packing solutions. Always start by determining the optimal packing flow velocity. Guidelines for determining optimal packing flow velocities for columns with adapters and fixed bed heights are given in the next section.

Determine the optimal packing flow velocity

The optimal packing flow velocity depends on temperature, column size and type, resin, and volume. As a result, the optimal packing flow velocity must be determined empirically for each individual system. To determine the optimal packing flow velocity, 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 amount 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 column instructions.

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

Packing the column

Follow the detailed instructions in the user manual supplied with your column.

Note: *It is recommended to perform CIP after packing, as column packing involves open handling of the resin.*

4 Evaluation of the packed column

Introduction

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 . The values are easily determined by applying a test sample such as 1% acetone solution to the column. Avoid colored compounds since they can interact with the resin.

Column efficiency testing

The calculated plate number depends on the test conditions and it must only be used as a reference value. It is important that conditions and equipment are kept the same so that results are comparable. Changes in solute, solvent, eluent, sample volume, flow rate, liquid pathway, temperature, etc., affect the results. For optimal results, the sample volume must be 1% to 3% of the column volume, and the flow velocity 20 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

Parameter	Description
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

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

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

$$\text{HETP} = L/N$$

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 (h) is calculated as follows:

$$h = \text{HETP} / d_p$$

where d_p = mean diameter (cm) of the particles

As a guideline, an acceptance value of < 3 for (h) can be used.

The peak must be symmetrical, with the asymmetry factor as close to 1 as possible. 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.

Calculate the peak asymmetry factor as follows:

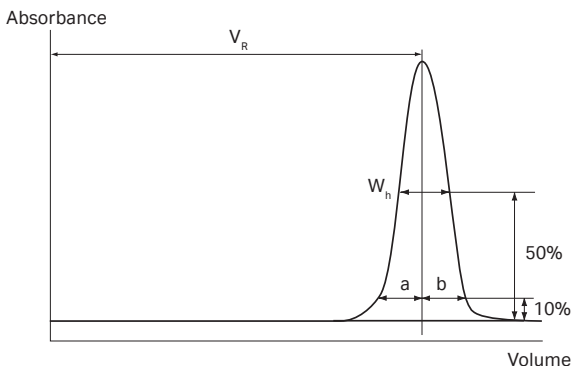
$$A_s = b/a$$

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 from which the HETP and A_s 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

W_h : 0.9

HETP: 0.024 cm

a : 0.90

b : 0.85

A_S : 0.94

5 Process optimization

For further information and details about process optimization and scale-up, refer to *Ion Exchange Chromatography: Principles and Methods (CY13983)*, available at cytiva.com.

6 Maintenance

Introduction

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 CIP, and before a chromatographic run, equilibrate with loading 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.

CIP

CIP is a procedure that removes impurities or contaminants such as lipids, endotoxins, nucleic acids, and precipitated or denatured proteins that remain in the packed column after regeneration. Regular CIP prevents the buildup of impurities or 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 impurities or contaminants present.

It is recommended to perform a CIP:

- before first-time use – especially after packing the column – or after long-term storage
- between cycles
- when an increase in back pressure or a reduction in column performance is observed
- to prevent potential cross-contamination or carry-over, when the same column is used for purification of different proteins or protein lots and batches

CIP protocol

A standard CIP protocol is described below.

Step	Action
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1	Wash with 0.5 column volumes of filtered 2 M NaCl. Contact time 10 to 15 min. Reversed flow direction.
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2	Wash with 1.0 M NaOH at 40 cm/h. Contact time 1 to 2 hours. Reversed flow direction. Wash with 2 to 4 column volumes of 0.5% nonionic detergent in 1 M acetic acid. Contact time 1 to 2 hours. Reversed flow direction.
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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.

Note: Consult local safety regulations to verify if safety precautions are required for the use of 70% ethanol or 30% isopropanol.

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. For more details, refer to Application note *Use of sodium hydroxide for cleaning and sanitization of chromatography resins and systems (CY13951)*.

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

Store unused resin in its 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.

7 Troubleshooting guide

High back pressure

Step	Action
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- | | |
|---|--|
| 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. |

Step	Action
3	Check if equipment used before and after the column is generating any back pressure. For example, valves, tubing, 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.
4	Check that there are no gaps between the adapter and the resin bed and no backmixing of the sample before application.
5	Check the efficiency of the column packing, see Chapter 4 Evaluation of the packed column, on page 10 .
6	Check for changes in the pretreatment of the sample.

Infections

Step	Action
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- | | |
|---|---|
| 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
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- | | |
|---|---|
| 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. Small amounts of air trapped on top of the bed, or between the adapter net and head, can be removed by pumping eluent or 20% ethanol in the opposite direction. Afterwards, check the efficiency of the packed bed (see [Chapter 4 Evaluation of the packed column, on page 10](#)) and compare the result with the original efficiency values.

8 Ordering information

All products are supplied in suspension in 20% ethanol.

Product	Pack size	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.

Related literature	Reference
Application note	
Use of sodium hydroxide for cleaning and sanitization of chromatography resins and systems	CY13951
Data files	
AxiChrom columns	CY10003
BPG columns	CY978
Chromaflo columns	CY13377
Sepharose Fast Flow ion exchange resins and prepacked column formats	CY13444
Handbook	
Ion Exchange Chromatography: Principles and Methods	CY13983

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



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