ANX Sepharose 4 Fast Flow (high sub)

Introduction

ANX Sepharose™ 4 Fast Flow (high sub) is a weak anion exchanger, designed to support the increasing need for ion exchange media with different selectivity and higher chemical stability at high pH. In addition, the pore size distribution of ANX Sepharose 4 Fast Flow (high sub) has been optimized for the separation of large proteins. ANX Sepharose 4 Fast Flow is a BioProcess™ Medium and is supported with a Regulatory Support File.

- **Different selectivity compared with established weak anion exchangers**
- **Applicable for separation of high molecular mass proteins**
- **Developed in co-operation with leading large-scale pharmaceutical manufacturers**

Characteristics

ANX Sepharose 4 Fast Flow (high sub) is a weak anion exchanger medium with tertiary amine groups attached to the base matrix Sepharose 4 Fast Flow via ether linkage and a hydrophilic spacer arm. The diethylaminopropylgroup is coupled in a mode that precludes formation of quaternary groups, which are typically seen on traditional DEAE media, resulting in a truly weak anion exchanger. The base matrix, Sepharose 4 Fast Flow, is a highly cross-linked, 4% agarose derivative with high chemical and physical stability and broad separation range.

$-CH_2CHOHCHH_2NH^+(CH_2CH_3)_2$

Fig. 2. The ion exchance group of ANX Sepharose 4 Fast Flow (high sub).

Fig. 1. ANX Sepharose 4 Fast Flow (high sub).

Type of ion exchanger	Weak anion
Ionic capacity	0.13-0.17 mmol Cl^-/ml medium
Matrix structure	Cross-linked 4% agarose
Exclusion limit	3×10^7 (globular proteins)
Particle form	Spherical, 45-165 µm
Mean particle size	90 µm
Chemical stability[1]	Stable in all commonly used aqueous buffers – 1.0 M NaOH – 20 and 70% ethanol – 8.0 M guanidine HCl – 8.0 M urea – 1.0 M acetic acid
Physical stability	Negligible volume variation due to changes in pH or ionic strength
Recommended pH	
working range[2]	3–10
Cleaning-in-place	2–14
Recommended working flow velocity	300-500 cm/h
Temperature stability	+4 – +40 °C
Storage	20% ethanol

[1] No significant change in ionic binding capacity after one year storage at room temperature
[2] The group is charged over this pH range

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

Different selectivity

ANX Sepharose 4 Fast Flow (high sub) displays different selectivity in comparison with the already established Fast Flow ion-exchangers and therefore offers an additional choice when selecting anion-exchange media (see Figure 3).

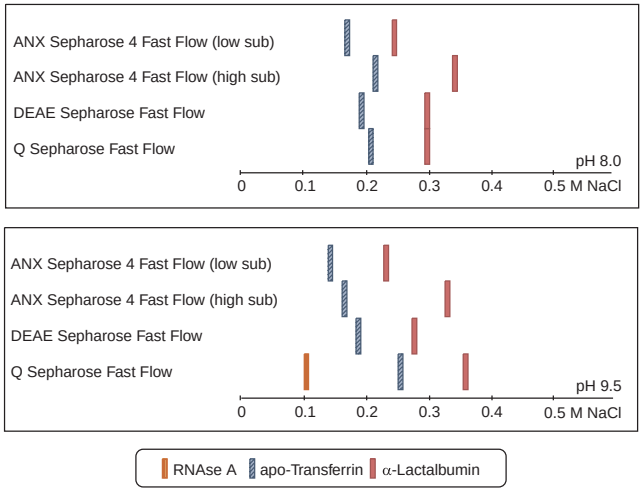

Fig. 3. Elution positions for RNAse A, apo-transferrin and α-lactalbumin on the four Fast Flow anion exchangers (ANX Sepharose 4 Fast Flow (high and low sub*), and Q and DEAE Sepharose Fast Flow) when run under identical conditions. **ANX Sepharose 4 Fast Flow (low sub) is a custom designed media product. Contact your local representative for more information.*

Separation of high molecular mass proteins

ANX Sepharose 4 Fast Flow (high sub) has larger pores than DEAE Sepharose Fast Flow which improves the dynamic binding capacity when separating larger molecules e.g thyroglobulin (M_r = 6.5×10^5). See Table 2.

Excellent chemical stability

Leakage of carbon from the base matrix is minimized, even at extreme pH, due to the improved coupling chemistry (see Figure 4). This feature allows the use of harsher cleaning-in-place (CIP) and sanitization protocols and also increases the lifetime of the medium.

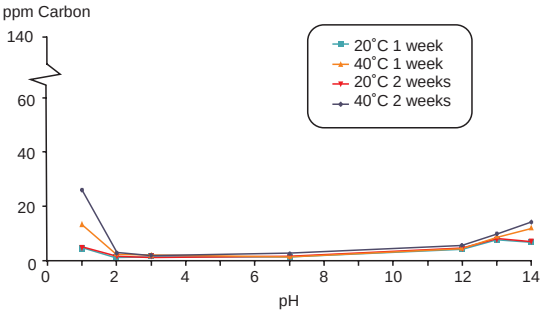

Fig. 4. The influence of pH, temperature, and time on the release of carbon from ANX Sepharose 4 Fast Flow (high sub).

Developed in co-operation with leading large-scale drug manufacturers

ANX Sepharose 4 Fast Flow (high sub) has been developed together with leading large-scale drug manufacturers. This has resulted in an adsorbent which is suitable for industrial processes, where parameters such as packing, scalability, low leakage, consistent performance and long lifetime are crucial.

Operation

ANX Sepharose 4 Fast Flow (high sub) ion exchanger is supplied as a suspension in 20% ethanol. Decant the 20% ethanol solution and replace with starting buffer before use. After packing, the medium should be equilibrated with approximately 5 bed volumes of starting buffer before use. Varying the pH, sample load, flow rate and the volume and shape of the pH or ionic strength gradient will affect resolution.

Regeneration

Regeneration of ANX Sepharose 4 Fast Flow (high sub) is easily carried out in the column, without the need for re-packing. After every run very tightly bound material is eluted using either high ionic strength (e.g. 1 M NaCl) or change in pH. The medium is re-equilibrated with starting buffer before each run.

Easy sanitization/cleaning-in-place

CIP is the on-column elimination and prevention of the build-up of very tightly bound, precipitated or denaturated substances that affect media capacity, flow properties and performance. In some applications, substances such as lipids

Anion exchange medium	Q_b BSA (mg/ml drained medium)	Q_b Thyroglobulin (mg/ml drained medium)
ANX Sepharose 4 Fast Flow (high sub)	43	5
DEAE Sepharose Fast Flow	47	1

[1] No significant change in ionic binding capacity after one year storage at room temperature

Table 2. Breakthrough capacity (Q_b) at 10% breakthrough of BSA and thyroglobulin for ANX Sepharose 4 Fast Flow (high sub) and DEAE Sepharose Fast Flow at 300 cm/h.

or denaturated proteins may remain in the column bed and not be eluted by the regeneration procedures. Therefore, a specific protocol has to be designed according to the type of contaminants known to be present in the feedstream. The frequency of CIP cycles depends on the nature and the condition of the starting material. Examples of CIP protocols are shown in Table 3.

Sanitization and sterilization

Sanitization is the use of chemical agents to inactivate microbial contaminants in the form of vegetative cells; it also helps to maintain a high level of both process hygiene and process economy. Like CIP, sanitization protocols are applied to chromatography systems and columns. Recommended sanitization and sterilization protocols for columns packed with ANX Sepharose 4 Fast Flow (high sub) are given in Table 3.

CIP protocol	
Ionically bound proteins	Wash with 0.5 column volumes of filtered 2 M NaCl. Contact time 10–15 min, reversed flow direction.
Precipitated proteins and hydrophobically bound proteins or lipoproteins	Wash with 1 M NaOH at 40 cm/h Contact time 1–2 h.
Lipids and very hydrophobic proteins	Wash with 70% ethanol, reversed flow at 40 cm/h for 1–2 h.
Sanitization	Wash with 0.5–1 M NaOH for 30–60 min.
Sterilization	Equilibrate the ion exchanger with Cl^- at pH 7. Autoclave the media at 121 °C for 30 min in buffer at pH 7.

[1] No significant change in ionic binding capacity after one year storage at room temperature

Table 3. CIP protocols, sanitization and sterilization procedures.

Easy scale-up

The performance of Sepharose Fast Flow media at all scales is well-documented: During the development of ANX Sepharose 4 Fast Flow (high sub) with large-scale pharmaceutical manufacturers it was shown that going from research-scale through pilot-scale and into production was a straightforward operation with maintained performance.

Applications

ANX Sepharose Fast Flow (high sub) for intermediate purification of large protein molecules

ANX Sepharose 4 Fast Flow (high sub) is the natural choice for the intermediate purification of large protein extracts, culture supernatants and other samples. The usual way of working with ANX Sepharose 4 Fast Flow (high sub) is to choose conditions so that the compounds of interest binds to the ion exchanger while most of the contaminants pass through. The components of interest can then be eluted in a small volume for further purification.

Column:	HiTrap™ ANX Sepharose 4 Fast Flow (high sub) 1 ml
Sample:	2 ml *E. coli* lysate clarified by centrifugation
Start buffer:	20 mM Tris™-HCl pH 7.4
Elution buffer:	20 mM Tris HCl, 0.5 M NaCl pH 7.4
Flow velocity	150 cm/h
Running parameters:	Sample application: 2 ml Wash: 10 ml start buffer Elution: 40 ml, linear gradient, 0-100% elution buffer
System:	ÄKTA™explorer 100 controlled by UNICORN™ 3.0

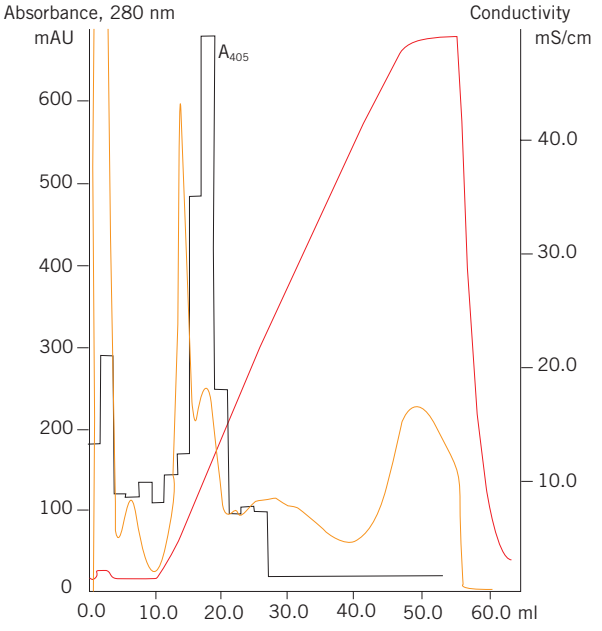

Fig. 5. Purification of alkaline phosphatase at 405 nm from *E. coli* lysate on HiTrap ANX Sepharose 4 Fast Flow (high sub).

Equipment

Recommended columns are given the table below.

Column	Inner diam. (mm)	Bed volume	Bed height (cm)
Lab Scale:			
HR 5/5	5	up to 1 ml	max. 5
HR 10/10	10	up to 8 ml	max. 10
HR 16/20	16	up to 30 ml	max. 15
HR 26/20	26	up to 80 ml	max. 15
XK 16/20	16	up to 20 ml	max. 10
XK 26/20	26	up to 53 ml	max. 10
Production scale:			
BPG™ 100/500	100	up to 2.4 l	max.30
BPG 200/500	200	up to 9.4 l	max.30
BPG 300/500	300	up to 21 l	max.30
BPG 450/500	450	up to 43 l	max.27
INdEX™ 100/500	100	up to 2.4 l	max. 30
INdEX 200/500	200	up to 9.4 l	max. 30
CHROMAFLOW™ 400*	400	18 l	15

* For large-scale separation, CHROMAFLOW columns with diameters greater than 400 mm are available as Custom Designed Columns. Please contact your local Amersham Biosciences representative for information.

Table 4. Recommended columns for ANX Sepharose 4 Fast Flow (high sub).

Storage

Store the medium in the salt form in a buffer containing a suitable anti-microbial agent e.g. 20% ethanol.
Recommended storage is at +4 – +30 °C.

Ordering information

Product	Pack size	Code No.
ANX Sepharose 4 Fast Flow (high sub)	25 ml	17-1287-10
	500 ml	17-1287-01
	5 l	17-1287-04
	10 l	17-1287-05
	60 l	17-1287-60

All bulk media products are supplied in suspension in 20% ethanol.

to order:

Asia Pacific Tel: +852 2811 8693 Fax: +852 2811 5251 **Australasia** Tel: +61 2 9894 5152 Fax: +61 2 9899 7511 **Austria** Tel: 01 576 0616 20 Fax: 01 576 0616 27 **Belgium** Tel: 0800 73 888 Fax: 03 272 1637 **Canada** Tel: 1 800 463 5800 Fax: 1 800 567 1008 **Central, East, South East Europe** Tel: +43 1 982 3826 Fax: +43 1 985 8327 **Denmark** Tel: 45 16 2400 Fax: 45 16 2424 **Finland** Tel: 09 512 3940 Fax: 09 512 1710 **France** Tel: 0169 35 67 00 Fax: 0169 41 9677 **Germany** Tel: 0761 4903 401 Fax: 0761 4903 405 **Italy** Tel: 02 27322 1 Fax: 02 27302 212 **Japan** Tel: 81 3 5331 9336 Fax: 81 3 5331 9370 **Latin America** Tel: +55 11 3667 5700 Fax: +55 11 3667 87 99 **Middle East and Africa** Tel: +30 (1) 96 00 687 Fax: +30 (1) 96 00 693 **Netherlands** Tel: 0165 580 410 Fax: 0165 580 401 **Norway** Tel: 2318 5800 Fax: 2318 6800 **Portugal** Tel: 01 417 7035 Fax: 01 417 3184 **Russian Federation** Tel: +7 (095) 232 0250,956 1137 Fax: +7 (095) 230 6377 **South East Asia** Tel: 60 3 724 2080 Fax: 60 3 724 2090 **Spain** Tel: 93 594 49 50 Fax: 93 594 49 55 **Sweden** Tel: 018 612 19 00 Fax: 018 612 19 10 **Switzerland** Tel: 01 802 81 50 Fax: 01 802 81 51 **UK** Tel: 0800 616 928 Fax: 0800 616 927 **USA** Tel: +1 800 526 3593 Fax: +1 800 329 3593

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