nProtein A Sepharose™ 4 Fast Flow

nProtein A Sepharose 4 Fast Flow is an affinity resin, designed for the purification of monoclonal and polyclonal antibodies at both laboratory and process scale. The resin is manufactured without using any animal-derived components.

The specificity of protein A is primarily for the Fc region of IgG, through which it binds, leaving the antigen combining sites free. In addition, one molecule of immobilized protein A has the capacity to bind at least two molecules of IgG.

Purified protein A is coupled to Sepharose 4 Fast Flow by the well established and proven CNBr method.

nProtein A Sepharose 4 Fast Flow is a BioProcess™ resin. BioProcess chromatography resins are developed and supported for production 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 submissions to regulatory authorities. BioProcess resins cover all purification steps from capture to polishing.

Table of Contents

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 Resin characteristics

Protein A is produced by fermenting a selected strain of *Staphylococcus aureus*.

The base matrix, Sepharose 4 Fast Flow, is a cross-linked, 4% agarose derivative with excellent kinetics, making it ideal for process scale applications. Purified protein A is coupled to Sepharose 4 Fast Flow by the well established CNBr method resulting in a very stable resin with very low leakage of protein A.

The total binding capacity of human IgG is ≥ 30 mg hIgG/mL resin. The dynamic capacity of chromatographic adsorbents is a function of the flow velocity used and it increases with decreasing flow velocity. In Figure 1 the flow velocity/capacity dependence is shown for four different flow velocities.

nProtein A Sepharose 4 Fast Flow has high chemical stability (Table 1). This enables it to withstand rigorous cleaning and sanitizing procedures, despite the relative labile nature of protein ligands. Even with strong chemical treatment, nProtein A Sepharose 4 Fast Flow performance is consistent over at least hundred process cycles.

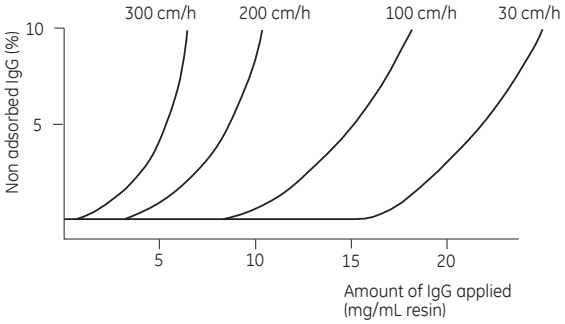

Fig 1. One example of how the capacity for human IgG depends on the flow velocity with nProtein A Sepharose 4 Fast Flow. The non-adsorbed IgG (%) was measured as a function of the amount applied to the column at 4 different flow velocities, 300, 200, 100, and 30 cm/h. Concentration of the applied sample: 0.33 mg IgG/mL. Column: HR 5/5 containing 1 mL of nProtein A Sepharose 4 Fast Flow. Buffer system: 0.1 M Na_2HPO_4, pH 7.0.

Table 1. Characteristics of nProtein A Sepharose 4 Fast Flow

Matrix	Cross-linked agarose, 4%, spherical
Particle size, d_{50V} [1]	~ 90 µm
Total binding capacity [2]	≥ 30 mg hIgG/mL resin
Recommended operating flow velocity	30 to 200 cm/h [3]
pH stability, operational [4]	3 to 9 [5]
pH stability, CIP [6]	3 to 10[5]
Chemical stability	Stable to commonly used aqueous buffers, 6 M guanidine-HCl, 70% ethanol, 3 M NaSCN, 0.1 M glycine (pH 3.0), 2% benzyl alcohol or 20 % ethanol.
Temperature stability	4°C to 40°C
Storage	20% ethanol, 2°C to 8°C

1 Median particle size of the cumulative volume distribution.

2 Protein in excess is loaded in 0.020 M NaH_2PO_4 at pH 7 on a 7.5/50 PEEK-column. The binding capacity is obtained by measuring the amount of eluted protein in 0.1 M Glycine at pH 3.

3 60 cm diameter, 20 cm bed height, at 20°C using buffer with the same viscosity as water.

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

5 pH below 3 is sometimes required to elute strongly bound IgG species. However, protein ligand may hydrolyze at pH below 2.

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

To maintain the same dynamic binding capacity when scaling-up the residence time must be the same as used when developing the binding conditions. Residence time is defined as:

$$\frac{\text{Bed height (cm)}}{\text{Flow velocity (cm/h)}}$$

It should also be noted that individual antibodies differ in their affinity to Protein A (see section 6, *Process optimization*).

2 Chromatography column packing guidelines

nProtein A Sepharose 4 Fast Flow is supplied in suspension in 20% ethanol. This resin is easy to pack since its rigidity allows the use of high flow velocities.

2.1 Recommended columns

Column	Inner diameter (mm)	Bed volume	Bed height (cm)
Lab-scale: XK 16/40	16	8 to 74 mL	max. 35
XK 26/40	26	32 to196 mL	max. 35
Production-scale: BPG variable bed, glass column	100 to 450	2.4 to 131 L	max. 83
Chromaflow™ variable bed columns	280 to 2000		

2.2 Preparing the resin

Let the resin settle overnight in a measuring cylinder. Prepare a 45%-55% (lab-scale) or 50%-70% (large-scale) slurry by adding 20% ethanol to the settled resin.

Note: *nProtein A Sepharose 4 Fast Flow is supplied preswollen in 20% ethanol.*

2.3 Packing nProtein A Sepharose 4 Fast Flow in XK columns

Materials

- nProtein A Sepharose 4 Fast Flow
- XK column
- XK packing connector XK 16 or XK 26
- XK column as packing tube
- 20% ethanol

Equipment

- Chromatography system, such as ÄKTA™ system, can be used for packing.
- Pressure monitor

Equilibrate all materials to room temperature.

Packing parameters

- Bed height 10 to 20 cm
- Slurry/packing solution: 20% ethanol
- Slurry concentration: 45% to 55%
- Step 1, consolidation velocity (cm/h): 45 cm/h (60 min)
- Step 2, packing velocity (cm/h): 160 cm/h (20 min

Table 2. Volumetric flow for different column sizes (mL/min).

Column size, i.d. (mm)	16	26	50
Step 1 (45 cm/h)	1.5	4	15
Step 2 (160 cm/h)	5.4	14	52

Packing instructions

Step	Action
1	Attach the packing connector together with another column (the second XK column act as packing tube) at the top of the column.
2	Wet the bottom filter by injecting 20% ethanol through the effluent tubing and attach filter and bottom piece on the column.
3	Assemble the column and packing tube vertically on a laboratory stand. Apply 20% ethanol 2 cm over the column bottom adapter and put a stop plug on the outlet.
4	Pour the medium slurry into the column and packing tube and if necessary top up carefully with 20% ethanol.
5	Connect the top adapter to the pump and prime the top adapter with packing solution.
6	Put the top adapter in the packing tube, sliding it down to the surface of the slurry and displacing the air under the adapter.
7	Pack the column with 20% ethanol at a constant flow (see *Table 2*, Step 1) and run for 60 min or until the resin bed is stable.
8	Increase the flow (*Table 2*, Step 2) and run for 20 minutes. ***Note:*** *The packing pressure in step 2 must not exceed 1.5 bar.*
9	Mark the bed height on the column.
10	Stop the pump, close the column outlet and remove the packing tube (if used).
11	Put the adapter in the column tube and adjust it down to approximately 2 cm above the bed surface with the o-ring untightened.
12	Tighten the O-ring and adjust the adapter down to the bed height noted in Step 10 with the inlet on top of the column open.

2.4 Determining optimal flow velocities for large-scale packing

The optimal packing flow velocity is dependent on column size and type, resin volume, packing solution and temperature. The optimal packing flow velocity must therefore 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 quantity of resin required per liter packed volume is approximately 1.15 liters sedimented resin.
2	Set up the column as for packing according to *2.1 Recommended columns, on page 6*.
3	Begin packing the resin at a low flow velocity (30 cm/h).
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, or the maximum flow velocity for the resin.
5	The maximum flow velocity 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 velocity. The optimal packing flow velocity/pressure is 70% to 100% of the maximum flow velocity/pressure.
6	Plot the pressure/flow velocity curve and determine the optimal packing flow rate.

The operational flow velocity/pressure should be < 70% of the packing flow velocity/pressure.

Note: *For more information on determining the optimal flow velocities for packing large-scale columns, see Application Note 29169455.*

2.5 Packing nProtein A Sepharose 4 Fast Flow in BPG columns

BPG columns

BPG columns are supplied with a movable adapter. They are packed by conventional pressure packing by pumping the packing solution through the chromatographic bed at constant flow rate (or back pressure)

Step	Action
1	Pour some water (or packing buffer) into the column. Make sure that there is no air trapped under the bottom net. Leave about 2 cm of liquid in the column.
2	Mix the packing buffer with the resin to form a 50% to 70% slurry. (Sedimented bed volume/slurry volume = 0.5 to 0.7.) Pour the slurry into the column. Insert the adapter and lower it to the surface of the slurry, making sure no air is trapped under the adapter. Secure the adapter in place.
3	Seal the adapter O-ring and lower the adapter a little into the slurry, enough to fill the adapter inlet with packing solution.
4	Connect a pump and a pressure meter and start packing at the predetermined packing flow velocity (or pressure). Keep the flow velocity (or pressure) constant during packing and check the pressure at the column inlet. Never exceed the pressure limit for column or resin.
5	When the resin has settled, mark the bed height on the column tube, close the bottom valve and stop the pump. The bed starts rising in the column. Loosen the O-ring and lower the adapter to about 0.5 to 1.0 cm from the resin surface.
6	Seal the O-ring, start the pump and continue packing. Repeat steps 5 and 6 until there is a maximum of 1 cm between resin surface and adapter when the resin has stabilized

Step	Action
7	Close the bottom valve, stop the pump, disconnect the column inlet and push the adapter down to approximately 3 mm below the mark on the column tube, without loosening the adapter O-ring. The packing solution will flush the adapter inlet. Remove any trapped air by pumping liquid from the bottom (after the inlet tubing and the bottom valve have been properly filled).

3 Evaluation of column packing

3.1 Intervals

Test the column efficiency to check the quality of packing. Testing should be done after packing and at regular intervals during the working life of the column and also when separation performance is seen to deteriorate.

3.2 Column efficiency testing

The best method of expressing the efficiency of a packed column is in terms of the height equivalent to a theoretical plate (HETP) and the asymmetry factor (A_s). These values are easily determined by applying a sample such as 1% acetone solution to the column. Sodium chloride can also be used as a test substance. Use a concentration of 0.8 M NaCl in water as sample and 0.4 M NaCl in water as eluent.

For more information about column efficiency testing, consult the application note *Column efficiency testing* (28937207).

Note: *The calculated plate number will vary according to the test conditions and it should only be used as a reference value. It is important that test conditions and equipment are kept constant so that results are comparable. Changes of solute, solvent, eluent, sample volume, flow velocity, liquid pathway, temperature, chromatography system, etc. will influence the results.*

3.3 Sample volume and flow velocity

For optimal column efficiency results, the sample volume should be approximately 1% of the column volume and the flow velocity 30 cm/h. If an acceptance limit is defined in relation to column performance, the column plate number can be used as one of the acceptance criteria for column use.

3.4 Method for measuring HETP and A_s

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

$$HETP = \frac{L}{N}$$

L = bed height (cm)
N = number of theoretical plates

$$N = 5.54 \times \left(\frac{V_R}{W_h}\right)^2$$

V_R = volume eluted from the start of sample application to the peak maximum
W_h = peak width measured as the width of the recorded peak at half of the peak height
V_R and W_h are in the same units

The concept of reduced plate height is often used for comparing column performance.

The reduced plate height, h, is calculated as follows:

$$h = \frac{HETP}{d_{50v}}$$

d_{50v} = Median particle size of the cumulative volume distribution (cm)

As a guideline, a value of < 3 is very good.

The peak should be symmetrical, and the asymmetry factor as close to 1 as possible (a typical acceptable range could be $0.8 < A_S < 1.8$).

A change in the shape of the peak is usually the first indication of bed deterioration due to excessive use.

Peak asymmetry factor calculation:

$$A_s = \frac{b}{a}$$

a = ascending part of the peak width at 10% of peak height
b = descending part of the 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.

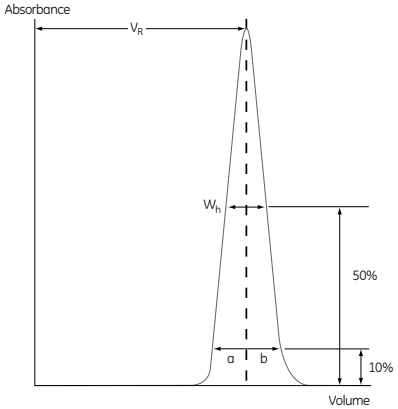

Fig 2. A typical test chromatogram showing the parameters used for HETP and A_s calculations.

4 Maintenance

For best performance from nProtein A Sepharose 4 Fast Flow over a long working life, follow the procedures described below.

4.1 Equilibration

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

4.2 Regeneration

After each separation cycle, regenerate the bed by washing with approximately 3 bed volumes of 0.1 M citrate buffer, pH 3, until the base line is stable, followed by at least 3 column volumes of binding buffer.

4.3 Cleaning-in-place

Cleaning-in-place, (CIP) is the removal of very tightly bound, precipitated or denatured substances from the purification system.If such contaminants are allowed to accumulate they may affect the chromatographic properties of the column. If the fouling is severe, it may block the column, increase back pressure and reduce flow rate. Regular CIP prevents the build up of these contaminants in the packing bed, and helps to maintain the capacity, flow properties and general performance.

Precipitated or denatured substances	Wash with 2 column volumes of 6 M guanidine hydrochloride [1], 10 mM NaOH [2], or 0.1 M H_3PO_4. Wash immediately with at least 5 column volumes of sterile filtered binding buffer at pH 7 to 8. Reversed flow direction.
Hydrophobically bound substances	Wash the column with 2 column volumes of a non-ionic detergent[1] (e.g., conc. 0.1%). Wash immediately with at least 5 column volumes of sterile filtered binding buffer at pH 7 to 8. Reversed flow direction.
or	Wash the column with 3 to 4 column volumes of 70% ethanol[1, 3]. Wash immediately with at least 5 column volumes of sterile filtered binding buffer at pH 7 to 8. Reversed flow direction. Apply increasing gradients to avoid air bubble formation when using high concentrations of organic solvents.

1 Apply for an approximate contact time of 10 minutes on the column.

2 Apply for an approximate contact time of 30 minutes on the column.

3 See Caution below!

CAUTION

70% ethanol can require the use of explosion-proof areas and equipment.

5 Sanitization

Sanitization reduces microbial contamination of the bed to a minimum.

- Equilibrate the column with a solution consisting of 2% hibitane digluconate and 20% ethanol. Allow to stand for 6 hours. Then wash with at least 5 column volumes of sterile binding buffer, or
- Equilibrate the column with a solution consisting of 0.1 M acetic acid and 20% ethanol. Allow to stand for 1 hour. Then wash with at least 5 column volumes of sterile binding buffer, or
- Equilibrate the column with 70% ethanol. Allow to stand for 12 hours. Then wash with at least 5 column volumes of sterile binding buffer.

Note: *Specific regulations may apply when using 70% ethanol since the use of explosion-proof areas and equipment may be required.*

CAUTION

70% ethanol can require the use of explosion-proof areas and equipment.

6 Process optimization

nProtein A Sepharose 4 Fast Flow is designed for purification of monoclonal and polyclonal antibodies. The primary specificity of protein A is for the Fc region of IgG, but it can also bind the Fab region through secondary sites. Binding affinity for the Fc region is usually stronger, which enables Fab or F(ab)2 to be fractionated from Fc.

The degree to which protein A binds to IgG varies with respect to both origin and antibody subclass (Table 3). This is an important consideration when developing the purification protocol. To achieve efficient capture of weakly bound antibodies, it is usually necessary to alter the formulation of the binding buffer in one of the following ways:

- Increasing pH titrates opposing histidyl residues in the binding sites of protein A and IgG. This reduces electrostatic repulsion between protein A and IgG, allowing an inhibited affinity interaction.
- Increasing salt concentration reduces electrostatic repulsion, and increases hydrophobic interactions.
- Reducing temperature has been reported to improve binding.

Table 3. Affinity of protein A for selected classes of monoclonal antibodies. This Table is compiled from a variety of sources and conditions, comparisons should be approximated.

Antibody	Affinity	Binding pH	Elution pH
Human			
IgG1	very high	6.0–7.0	3.5–4.5
IgG2	very high	6.0–7.0	5–4.5
IgG3	low-none	8.0–9.0	≤7.0
IgG4	low-high	7.0–8.0	3.0–6.0
Mouse			
IgG1	low	8.0–9.0	5.5–7.5
IgG2a	moderate	7.0–8.0	4.5–5.5
IgG2b	high	≈7.0	3.5–4.5
IgG3	low-high	≈7.0	4.0–7.0
Rat			
IgG1	very low	>9.0	7.0–8 .0
IgG2a	low-none	>9.0	≤8.0
IgG2b	low-none	>9.0	≤8.0
IgG2c	very low	8.0–9.0	≤7.0

6.1 Operation

Stage	Description
1	Pack the column as described in chapter *2 Chromatography column packing guidelines*.
2	Wash the packed bed with at least 3 column volumes of binding buffer to remove preservative.
3	Filter the sample through a 0.2 to 0.45 µm filter and load the sample (the sample pH should be the same as the binding buffer pH).
4	Wash the resin with binding buffer until the baseline is stable.
5	Elute the sample in normal or reversed flow direction.

6.2 Optimizing conditions

Optimizing the method for antibody fractionation with nProtein A Sepharose 4 Fast Flow is best done as follows:

- Pack and equilibrate a small column (or use a HiTrap™ Protein A) in high salt conditions.
- Load a small sample of antibody.
- Elute in a linear pH gradient.

Bed dimensions:	1×1.25 cm (1 mL)
Start buffer:	0.05 M boric acid, 0.05 M Na_2PO_4, 0.05 M Na citrate, 4.0 M NaCl, pH 9.0
Elution buffer:	0.05 M boric acid, 0.05 M Na_2PO_4, 0.05 M Na citrate, pH 3.0
Sample:	10 mL filtered cell culture supernatant, equilibrated to buffer A by addition of dry buffers salts and titration with NaOH
Flow velocity (Flow rate):	75 cm/h (1 mL/min)
Conditions:	Equilibrate column with 5 column volumes (CV) of starting buffer, apply sample, wash 2 CV starting buffer, elute with a 10 CV linear gradient beginning at 100% start buffer and ending at 100% elution buffer, strip with 5 CV 100% elution buffer.
Peak 1:	Bovine IgG
Peak 2:	Monoclonal IgG

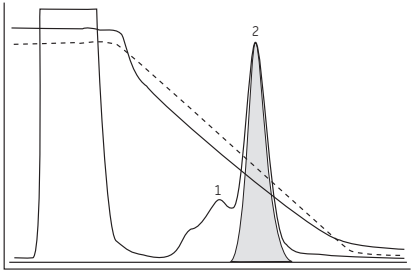

Fig 3. Analytical scale linear pH gradient elution of mouse IgG2a from nProtein A Sepharose 4 Fast Flow.

Antibodies that elute early can be eluted using linear gradients since this provides the best and most reproducible fractionation from contaminating antibodies, see Figure 6.

For antibodies that bind more strongly to protein A, step gradient elution is recommended since this shortens the time that the antibody is exposed to the elution conditions, see Figure 7.

Bed dimensions:	5.0×6.3 cm (125 mL)
Starting buffer:	0.05 M boric acid, 0.05 M Na_2PO_4, 0.05 M Na citrate, 4.0 M NaCl, pH 9.0
Elution buffer:	0.05 M boric acid, 0.05 M Na_2PO_4, 0.05 M Na citrate, pH 3.0
Sample:	125 ml filtered ascites, equilibrated to start contitions by direct addition of dry buffers salts and titration with NaOH.
Flow velocity (Flow rate):	75 cm/h (25 mL/min)
Conditions:	Equilibrate column with 5 column volumes (CV) of starting buffer, apply sample, wash 2 CV starting buffer, elute with a 10 CV linear gradient beginning at 100% start buffer and ending at 100% elution buffer, strip with 5 CV elution buffer

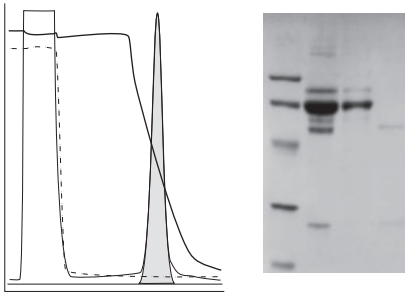

Fig 4. Preparative scale step pH gradient elution of a mouse IgG, from nProtein A Sepharose 4 Fast Flow. Lane 1: LMW stds. Line 2: ascites. Line 3: flow-through. Line 4: purified IgG.

Be sure that the antibody is stable under the elution conditions selected.

If there is any doubt, titrate the antibody fraction to neutrality immediately upon elution in order not to lose biological activity.

To accomplish this, prefill the fraction vessels to 5% to 10% of the intended fraction volume with a buffer concentrate at about pH 7.5 (for example 1 M Tris-HCl or 1 M sodium phosphate).

Another frequent practice to reduce exposure of the antibody to harsh conditions is to reverse the flow direction during elution. This also elutes the antibody in a more concentrated fraction.

Removal of leached protein A

Leakage of protein A from nProtein A Sepharose 4 Fast Flow is generally low. When such a low level of leakage is of concern e.g., in purification of monoclonal antibodies for clinical applications, a step has to be added to eliminate the possibility of contamination by leached protein. Leached protein A can be removed from the final product in several ways:

- Size exclusion chromatography on Superdex™ 200 prep grade can be performed in low pH conditions in which the particular monoclonal is known to be fully dissociated from protein A. However, exposure of the antibody to low pH conditions increases the risk of loss of activity and aggregation.
- Aggregates of protein A-IgG can be removed by chromatography under moderate pH and conductivity conditions (although it may be difficult to achieve quantitative fractionation).

Both examples are illustrated in Figure 8.

Column:	XK 16/70, bed height 60 cm (120 mL)
Sample:	5 mL protein A purified antibody 0.5 mg/mL
Flow velocity (Flow rate):	50 cm/h (1.7 mL/min)
Buffer (run A):	0.1 M Na_2PO_4, 0.3 M NaCl, pH 7.0
Buffer (run B):	0.1 M Na acetate, 0.3 M NaCl, pH 4.1

Protein A is indicated by the black bar.

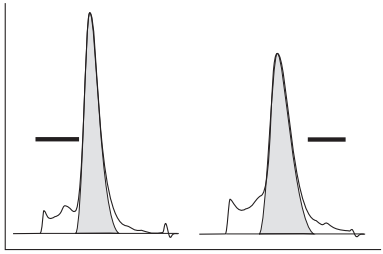

Fig 5. Removal of leached protein A from mouse IgG2b monoclonal by size exclusion chromatography on Superdex 200 prep grade. Note the higher proportion of aggregates and the broader peak in the sample run at low pH.

- Cation exchange chromatography is an effective tool for removing residual protein A, especially when the particular monoclonal has strong cation exchange binding characteristics. Perform the run at a pH in which the antibody is known to dissociate from protein A, see Figure 9. Protein A binds poorly to cation exchangers and elutes early in the gradient. The relative speed, capacity and scalability of thistechnique makes it preferable to size exclusion chromatography at low pH.

Bed dimensions:	1×8 cm (8.5 mL)
Start buffer:	0.05 M MES, pH 5.6
Elution buffer:	0.05 M MES, 1.0 M NaCl, pH 5.6
Sample:	6 mL protein A purified antibody 0.5 mg/mL
Flow velocity (Flow rate):	200 cm/h (4.2 mL/min)
Conditions:	Equilibrate column with CV of start buffer, apply sample, wash 5 CV starting buffer, elute with a 10 CV linear gradient beginning at 100% start buffer and ending at 25% elution buffer, strip with 5 CV 100% elution buffer.

Protein A is indicated by the black bar.

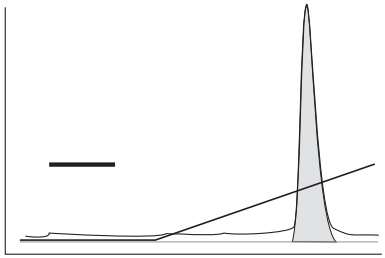

Fig 6. Removal of leached protein A from mouse IgG monoclonal by cation exchange chromatography on S Sepharose High Performance.

- Anion exchange works best with antibodies that are retained weakly on anion exchangers. Since protein A binds strongly to anion exchangers, complexes of IgG-protein A are retained more strongly than non-complex antibodies. These complexes do not form separate peaks, but often exhibit a trailing shoulder. To determine the ability of anion exchange chromatography to remove complex protein A, equilibrate the column to 0.05 M Tris, pH 8.6, apply sample and elute in a linear gradient ending at 0.25 M NaCl (0.05 M Tris, pH 8.6). Collect fractions across the antibody peak and screen for protein A.

6.3 Scaling-up

After optimizing the antibody fractionation at laboratory scale, the process can be scaled up. For this, some parameters will change while others remain constant.

Stage	Description
1	First consider the residence time. This should not be shorter than the time established in the small scale experiments.
2	Select bed volume according to required binding capacity.
3	Select column diameter to obtain a bed height of 5 to 30 cm so that high flow velocities can be used. Max. flow velocity is approx. inversely proportional to the bed height. Expect to operate at no more then 70% of the max. flow velocity.
4	Keep sample concentration and the ratio of gradient volume/resin volume constant.

The larger equipment needed when scaling-up may cause some deviations from the optimized method at small scale. In such cases, check the buffer delivery system and monitoring system for time delays or volume changes. Different lengths and diameters of outlet pipes can cause zone spreading on larger systems.

7 Troubleshooting

Problem	Solution	
High back-pressure	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, nets etc., according to the column instruction manual.
Unexpected chromatographic results	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 bed, or back-mixing of the sample before application.
	5	Check the efficiency of the column packing, see chapter 3.
	6	Check if there have been any changes in the pretreatment of the sample.
Infections	1	Check the connections and prefilters.
	2	Check the in-going components such as buffers, sample components, etc.
	3	Check that the column has been properly sanitized.

Problem	Solution
Trapped air	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, repack the column. 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 *Evaluation of column packing*, and compare the result with the original efficiency values.

8 Storage

All bulk resins products are supplied in suspension in 20% ethanol. For longer periods of storage, keep the medium at 2°C to 8°C in a suitable bacteriostat, for example 20% ethanol. The medium must not be frozen.

9 Ordering information

Product	Quantity	Product code
nProtein A Sepharose 4 Fast Flow	5 mL	17528001
	25 mL	17528004
	200 mL	17528002
	1 L	17528003
	5 L	17528005
	10 L	17528006

Reference literature

Adner, N., & Sofer, G. (1993) *BioPharm vol 7* (3), 44–48
Füglistaller, P. (1989) *J. Immunol. Methods 124*, 171–177

Griffiths, H., Crisp, F., Henwood, & Rowland, D. (1993) *J. Cellul. Biochem.* Protein Purification and Engineering, 22nd Keystone Symposia, Wiley-Liss.

For general advice on optimization, scaling-up and other aspects relating to process chromatography we recommend:
Process chromatography – A practical guide. Eds., G.K. Sofer & L.E. Nyström, Academic Press, London, 1989

10 Further information

For further informations visit www.gelifesciences.com or contact your local GE representative.

For local office contact information, visit
www.gelifesciences.com/contact

GE Healthcare Bio-Sciences AB
Björkgatan 30
751 84 Uppsala
Sweden

www.gelifesciences.com

GE Healthcare Europe GmbH
Munzinger Strasse 5, D-79111 Freiburg, Germany

GE Healthcare UK Limited
Amersham Place, Little Chalfont, Buckinghamshire, HP7 9NA, UK

GE Healthcare Bio-Sciences Corp.
100 Results Way, Marlborough, MA 01752, USA

HyClone Laboratories, Inc.
925 W 1800 S, Logan, UT 84321, USA

GE Healthcare Japan Corporation
Sanken Bldg. 3-25-1, Hyakunincho Shinjuku-ku, Tokyo 169-0073, Japan

71502785 AD 12/2017 a56