rProtein A Sepharose™ Fast Flow

rProtein A Sepharose Fast Flow is a part of the BioProcess™ resin product portfolio.

rProtein A Sepharose Fast Flow resin is an affinity resin developed for purification of monoclonal and polyclonal antibodies in both research and industry applications.

The specificity of Protein A is primarily for the Fc region of IgG and leaving the antigen binding sites of the antibody free. The resin can also bind the Fab region of the antibody through the secondary sites. There are differences between the binding affinities for Fc and Fab, usually Fc binding is stronger, which can provide a means of fractionating Fab or F(ab)2 from the Fc region.

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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 BioProcess chromatography resins

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

2 Characteristics of rProtein A Sepharose Fast Flow

Introduction

The recombinant Protein A used in the manufacture of rProtein A Sepharose Fast Flow is specially engineered to give very high binding capacities. It is produced in *E. coli* and purified by a multi-step chromatographic procedure. The purification does not involve the use of IgG or any other proteins. The purified recombinant Protein A is tested according to established specifications before being released for the manufacture of rProtein A Sepharose Fast Flow.

The base matrix, Sepharose 4 Fast Flow, is a cross-linked, 4% agarose derivative with excellent chemical and physical stabilities, making it ideal for process scale applications.

Purified recombinant protein A is coupled to Sepharose 4 Fast Flow by a technique that generates a stable thioether linkage between protein A and the base matrix. The coupling technique is optimized to give a high binding capacity for IgG. The dynamic capacity of chromatographic adsorbents is a function of the flow velocity used and it increases with decreasing flow velocity. An example of the flow velocity/capacity dependence for three different flow velocities is shown in Figure 1.

The high chemical stability of rProtein A Sepharose Fast Flow enables it to withstand rigorous cleaning and sanitizing procedures, despite the relatively labile nature of protein ligands. For more resin characteristics see Table 1.

Bed dimensions:	1.6 × 10 cm (20 mL)
Binding buffer:	20 mM sodium phosphate, pH 7.0
Sample:	hIgG, 0.5 mg/mL
Flow velocity:	a) 100 cm/h; b) 300 cm/h; c) 450 cm/h
Breakthrough capacity:	a) 48 mg/mL resin b) 38 mg/mL resin c) 24 mg/mL resin

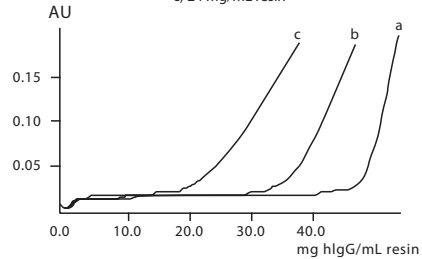

Fig 1. Example of flow velocity/capacity dependence for rProtein A Sepharose Fast Flow. Breakthrough capacity for hIgG was determined at 5% breakthrough for three different flow velocities.

Figure 2 shows typical pressure/flow velocity characteristics of the resin.

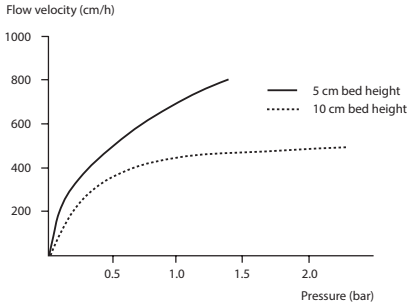

Fig 2. rProtein A Sepharose Fast Flow, typical pressure/flow velocity characteristics. Determined in a BPG™ 200/500 column (i.d. 200 mm) using water as the mobile phase at 20°C.

Table 1. Characteristics of rProtein A Sepharose Fast Flow

Matrix	Cross-linked agarose, 4%, spherical
Particle size d_{50v} [1]	~ 90µm
Ligand	Recombinant protein A (*E. coli*)
Coupling chemistry	Epoxy
Dynamic binding capacity, QB10 [2]	~ 35 mg human IgG/mL resin
Chemical stability	Stable to commonly used aqueous buffers, 6 M guanidine hydrochloride, 2% benzyl alcohol, 1 mM NaOH (pH 11), 0.1 M sodium citrate/HCl (pH 3), 20% ethanol
pH stability, operational [3]	3 [4] to 10
pH stability, CIP [5]	3 [4] to 12 [6]
Pressure/flow characteristics [7]	150-250 cm/h at < 0.1 MPa in a XK 50/60 column with 5 cm diameter and 25 cm bed height, and using buffers with the same viscosity as water at 20°C.
Temperature stability	2°C to 40°C
Delivery condition	20% ethanol
Storage	20% ethanol, 2°C to 8°C

1 Median particle size of the cumulative volume distribution.

2 Dynamic binding capacity at 10% breakthrough by frontal analysis at a mobile phase velocity of 100 cm/h in a 7.5/50 PEEK-column at 5 cm bed height (3 min residence time) for human IgG in 0.020 M NaH_2PO_4, pH 7.0.

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

4 pH below 3 is sometimes required to elute strongly bound IgG species. The protein ligands may hydrolyze at pH below 2.

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

6 Adding reducing agent e.g., 100 mM 1-thioglycerol followed by 15 mM NaOH is among the most efficient CIP for rProtein A Sepharose Fast Flow.

7 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. The Pressure/flow test is performed on the base matrix.

3 Packing lab-scale columns

Recommended columns

Table 1. Recommended lab-scale columns

Name	Description
HiScale 16/20	i.d. 16 mm, bed volumes up to 40 mL, bed heights up to 20 cm.
HiScale 16/40	i.d. 16 mm, bed volume 16 to 80 mL, bed heights 8 to 40 cm.
HiScale 26/20	i.d. 26 mm, bed volume 106 mL, bed height up to 20 cm.
HiScale 26/40	i.d. 26 mm, bed volume 69 to 212 mL, bed height 13 to 40 cm.
HiScale 50/20	i.d. 50 mm, bed volume up to 393 mL, bed height up to 20 cm.
HiScale 50/40	i.d. 50 mm, bed volume 274 to 785 mL, bed height 14 to 40 cm.
Tricorn™ 10/100	i.d. 10 mm, bed volumes up to 8.5 mL at bed height up to 11 cm.
XK 16/20	i.d. 16 mm, bed volumes up to 30 mL at bed height up to 15 cm.
XK 26/20	i.d. 26 mm, bed volumes up to 80 mL at bed height up to 15 cm.

Packing procedure

Ideally, rProtein A Sepharose Fast Flow is packed at a constant pressure of approximately 0.1 MPa (1 bar).

Materials needed

- rProtein A Sepharose 4 Fast Flow
- XK or HiScale column
- XK packing connector (XK 16 or XK 26) or HiScale packing tube
- XK column as packing tube (exclude when packing HiScale)
- 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

Step	Action
1	Assemble the packing tube for HiScale or packing connector together with another column (the second column act as packing tube) for XK columns, 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 to 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 resin 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.

Step	Action
6	Assemble 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 minutes or until the resin bed is stable.
8	Increase the flow (see Table 2, Step 2) and run for 20 minutes. ***Note:*** *The packing pressure in step 2 should not exceed 1.5 bar.*
9	Mark the bed height on the column.
10	Stop the pump, close the column outlet and dismount the packing tube (if used).
11	Assemble 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.

4 Packing process-scale columns

Recommended columns

Table 3. Recommended process-scale columns

Name	Description
BPG 100/500	(100 mm i.d.) for bed volumes up to 2.4 liters at a maximum bed height of 30 cm.
BPG 200/500	(200 mm i.d.) for bed volumes up to 9.4 liters at a maximum bed height of 30 cm.
BPG 300/500	(300 mm i.d.) for bed volumes up to 21 liters at a maximum bed height of 30 cm.
BPG 450/500	(450 mm i.d.) for bed volumes up to 43 liters at a maximum bed height of 27 cm.

General packing recommendations

Columns can be packed in different ways depending on the type of column and equipment used. Always read and follow the relevant column instruction manual carefully. How well the column is packed has a major effect on the result of the separation. Therefore, it is very important to pack and test the column according to the recommendations.

Start the packing procedure by determining the optimal packing flow velocity.

Determining optimal packing flow velocity for columns with adapters and fixed bed heights

The optimal packing flow velocity is dependent on column size and type, bed height, 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 the resin needed for the slurry. The quantity of resin required per liter packed bed is approximately 1.15 liters sedimented resin. ***Note:*** *Calculating exact amount of the resin is especially important for columns with fixed bed heights.*
2	Prepare the column exactly as for column packing.
3	Start packing the column at a low flow velocity (e.g., 30% of the expected max flow velocity) and record the flow velocity and back pressure when the bed is packed and the pressure has stabilized.
4	Increase the flow velocity in small steps and record the flow velocity and pressure at each step after the pressure has stabilized.
5	Continue recording flow and pressure until the maximum flow velocity is reached, that is when the flow velocity levels off at a plateau (indicating bed compression), or when the pressure reaches the pressure specification of the used column.
6	Plot pressure against flow velocity as indicated in Figure 2. The optimal packing flow velocity/pressure is 70% to 100% of the maximum flow rate/pressure. ***Note:*** *The operational flow velocity/pressure should be < 70% of the packing flow velocity/pressure.*

Packing procedure for BPG column

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 a constant flow velocity (or back pressure).

Step	Action
1	Pour 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	Prepare a 50% to 70% slurry by mixing the packing buffer with the resin and pour the slurry into the column. ***Note:*** *Sedimented bed volume/slurry volume = 0.5 to 0.7*
3	Insert the adapter and lower it to the surface of the slurry. Secure the adapter in place. ***Note:*** *Check that no air is trapped under the adapter*
4	Seal the adapter O-ring and lower the adapter a little into the slurry, enough to fill the adapter inlet with the packing solution.
5	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. ***Note:*** *Never exceed the pressure limit for column or the resin.*
6	When the bed is stabilized, close the bottom valve and stop the pump. *Result*: The bed starts rising in the column.
7	Loosen the O-ring and lower the adapter 0.5 to 1.0 cm above the bed surface.
8	Seal the O-ring, start the pump and continue packing. Mark the bed height on the column tube.

Step	Action
9	Repeat steps 6 to 9 until there is a gap of 1 cm between bed surface and the adapter when the bed is stabilized.
10	Close the bottom valve and stop the pump.
11	Disconnect the column inlet and, without loosening the adapter O-ring, push the adapter down to approximately 3 mm below the mark on the column tube.
	Result: The packing solution flushes the adapter inlet.
12	When the inlet tubing and the bottom valve are properly filled, remove any trapped air by pumping liquid from the bottom.

5 Packing evaluation

Introduction

The packing quality needs to be checked by column efficiency testing. The test must be done after the packing and at regular intervals during the working life of the column and also when the separation performance is seen to deteriorate.

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). The values are easily determined by applying a test sample such as 1% acetone solution or sodium chloride to the column.

Note:

Use a concentration of 0.8 M NaCl in water as sample and 0.4 M NaCl in water as eluent.

The calculated plate number is depended on the test conditions and must only be used as a reference value. It is important that the test conditions and the equipment are the same so that the results are comparable.
Changing the solute, solvent, eluent, sample volume, flow velocity, influences the results.

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

Sample volume and flow velocity

For optimal column efficiency results, the sample volume must be approximately 2.5 % 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 one of the acceptance criteria for the column use.

Method for measuring HETP and A_s

Method parameters:

Parameter	Description
Sample volume	2.5% of the bed volume
Sample concentration	1.0% (v/v) acetone
Flow velocity	15 cm/h
UV	280 nm, 1 cm, 0.1 AU

To avoid dilution of the sample, apply it as close to the column inlet as possible and calculate HETP and A_s from the UV curve (Fig. 3) (or conductivity curve) as follows:

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

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

N is calculated as follows:

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

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.

To facilitate comparison of column performance the concept of reduced plate height is often used. Calculate the reduced plate height as follows:

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

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

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

The peak must be symmetrical with an asymmetry factor as close to 1 as possible. A change in the shape of the peak is usually the first indication of bed deterioration due to excessive use. A typical acceptable range is $0.8 < A_s < 1.8$.

Calculate the peak asymmetry as follows:

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

a = 1st half peak width at 10% of peak height.
b = 2nd half peak width at 10% of peak height.

Figure 3 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 6 Fast Flow
Bed height: 57.5 cm
Bed volume: 40.6 liters
Eluent: Distilled water
Sample: 1.05 liters (1% acetone)
Flow velocity: 19 cm/h
$W_e = 18.7$
$W_h = 0.9$
HETP = 0.024 cm
a: 0.90
b: 0.85
A_s: 0.94

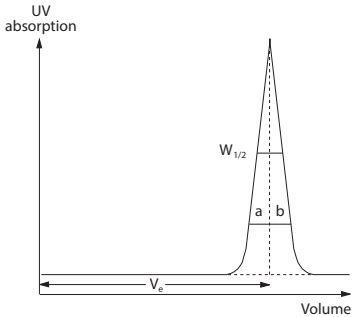

Fig 3. UV trace for acetone in a typical test chromatogram showing the HETP and A_s calculations.

6 Method design

The primary aim of method design is to establish conditions that enables binding the highest amount of target molecule, in the shortest time and with the highest product recovery.

Specificity and affinity

The degree to which protein A binds to IgG varies with respect to both origin and antibody subclass. This is an important consideration when developing the purification protocol.

Table 4. Affinity of protein A for selected classes of monoclonal antibodies. This table is compiled from a variety of sources. Comparisons should be understood to be approximate since they are derived from runs conducted under a variety of conditions.

Antibody	Affinity	Binding pH	Elution pH
Human			
IgG_1	Very high	6.0 to 7.0	3.5 to 4.5
IgG_2	Very high	6.0 to 7.0	3.5 to 4.5
IgG_3	Low-none	8.0 to 9.0	≤ 7.0
IgG_4	Low-high	7.0 to 8.0	3.0 to 6.0
Mouse			
IgG_1	Low	8.0 to 9.0	4.5 to 6.0
IgG_{2a}	Moderate	7.0 to 8.0	3.5 to 5.5
IgG_{2b}	High	~ 7.0	3.0 to 4.0
IgG_3	Low-high	~ 7.0	3.5 to 5.5

Method screening

The affinity of rProtein A Sepharose Fast Flow for antibodies of different species, classes and subclasses varies. The initial screening should be conducted under conditions that bind the largest diversity of antibodies that reveal the relationship between the target antibody and possible contaminating antibodies.

An effective way of mapping antibody behavior on rProtein A Sepharose Fast Flow is to bind them at high pH and high salt conditions, then elute them in a reducing linear salt/pH gradient.

It is important to make certain that the antibody is stable under the elution conditions. Titrate the antibody fraction to neutrality, immediately upon elution in order not to lose the biological activity.

Recommended screening conditions

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

Suitable buffer	Experimental condition
Buffer A: 20 mM sodium phosphate, 150 mM NaCl, pH 7	• Equilibrate the column with 10 column volumes of buffer A • Apply a small sample of antibody • Wash the column with 5 column volumes of buffer A • Elute the column with a linear gradient of 10 column volumes to 100%
Buffer B: Either 50 mM sodium phosphate pH 3.0 or 50 mM sodium citrate, pH 3.0	• Collect fractions into titrating diluent (e.g., 1.0 M Tris-HCl, pH 8.0) so that the diluent volume equals 5% of the programmed fraction volume • Regenerate the column with 5 to 10 column volumes of 100% buffer B • Re-equilibrate the column with buffer A

Conditions can be subsequently modified to provide the best purification performance. Due to the natural diversity of antibodies, binding and elution conditions must be optimized for the antibody to be purified.

Binding condition

To achieve efficient capture of the target antibody, it is often necessary to enhance the binding strength by 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.

- increases hydrophobic interactions.

By reducing the temperature reported, the dynamic binding capacity will most likely decrease.

High salt concentration and high pH often increase dynamic binding capacity. On the other hand, by decreasing salt concentration and/or pH during binding it is possible to avoid binding contaminating antibodies. This improves the selectivity and thereby increases the dynamic binding capacity.

The balance between selectivity and capacity must be defined with respect to the nature of the feed, (i.e., presence of contaminating antibodies and the purity requirement in the eluted product).

For some antibodies, good binding can be achieved without enhancing the binding strength. For low affinity antibodies, for example mouse IgG1, it is usually necessary to add up to 4 M NaCl to the binding buffer and to the feed material to achieve an efficient binding.

Elution condition

When optimizing elution conditions, determine the highest pH that allows efficient desorption of antibody from the column to prevents denaturation of sensitive antibodies due to exposure to low pH values.

Stepwise elution (Fig 4) is often preferred in large scale applications since it is technically simpler than elution with continuous gradients. It also allows the target monoclonal antibody to be eluted more concentrated resulting in less buffer consumption and shorter cycle times.

The linear gradient used in the screening experiment reveals the relative binding requirements of the target antibody relative to the contaminating antibodies. Linear gradient elution may be feasible for scale up and provides the best and most reproducible fractionation from contaminating antibodies.

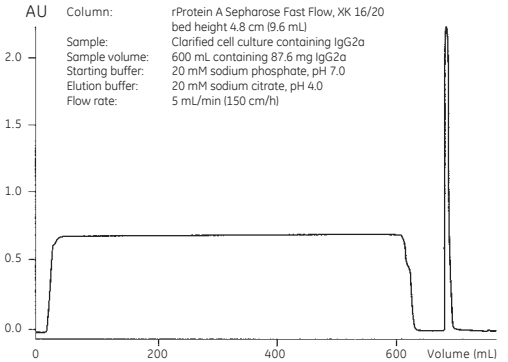

Fig 4. Purification of a monoclonal IgG2a from clarified cell culture on rProtein A Sepharose Fast Flow.

7 Optimization

Throughput optimization

When optimizing for highest throughput and productivity, it is necessary to define the highest sample load in the shortest sample application time with the most acceptable loss in the product recovery.

The dynamic binding capacity for the target antibody should be determined by frontal analysis using real process feedstock. The dynamic binding capacity is a function of the linear flow velocity applied during sample application, the breakthrough capacity must be defined over a range of different flow velocities. The optimal flow velocity gives the highest throughput in terms of amount of antibody processed per time unit and volume of the resin.
Example of breakthrough profiles at different flow velocities are shown in Figure 1.

Removal of leached Protein A from final product

Leakage of protein A from rProtein A Sepharose Fast Flow is generally low. In many monoclonal applications, the leached protein A must be eliminated from the final product. There are many chromatographic solutions for the removal of the leached Protein A. Below are three suggested solutions:

Method	Description
Size exclusion chromatography	Separation is performed under moderate pH conditions. The large IgG-Protein A complexes that are formed elute early from the column (Fig 5).
Cation exchange chromatography	Used especially when the particular monoclonal has strong cation exchange binding characteristics. The run is conducted at a pH in which the antibody is known to dissociate from Protein A. Protein A binds poorly to cation exchangers and will pass unretained or elute early in the gradient (Fig 6).
Anion exchange chromatography	It is best suited to antibodies that are weakly retained on anion exchangers. Because of the strong anion exchange binding characteristics of protein A, Protein A-IgG complexes tend to be more strongly retained than non-complex antibodies (Fig 7). Protein A-IgG complexes do not generally form separate peaks, but often exhibit a trailing shoulder. To determine the ability of anion exchange chromatography to remove complex Protein A follow the steps below: • Equilibrate the column with 20 mM Tris-HCl, pH 8.5. • Apply sample. • Elute in a linear gradient ending at 0.25 M NaCl (20 mM Tris-HCl, pH 8.5). • Collect fractions across the antibody peak and screen for Protein A.

Column: HiLoad 16/60 Superdex 200prep grade, bed height 60 cm (120 mL)
Sample: Purified antibody (14 mg) spiked with recombinant protein A (0.36 mg)
Sample volume: 4.8 mL
Flow velocity: 60 cm/h
Buffer: 20 mM sodium phosphate, 0.15 M NaCl, pH 7.0

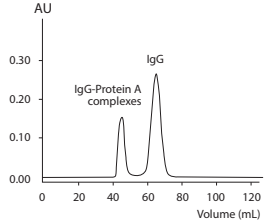

Fig 5. Removal of IgG-Protein A complex from mouse IgG_{2a} by size exclusion chromatography on Superdex™ 200 prep grade.
Recombinant Protein A was spiked into mouse IgG_{2a} previously purified on rProtein A Sepharose Fast Flow.

Column: HiTrap SP (1 mL)
Sample: Purified antibody (0.61 mg) spiked with recombinant protein A (1.8 mg)
Buffer A: 20 mM sodium citrate, pH 5.2
Buffer B: 20 mM sodium citrate, 1.0 M NaCl, pH 5.2
Flow velocity: 150 cm/h
Gradient: 0-45 %B, 15 column volumes

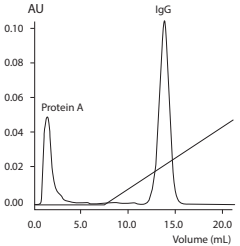

Fig 6. Removal of Protein A from mouse IgG_{2b} by cation exchange chromatography on HiTrap™ SP HP.
Recombinant Protein A was spiked into mouse IgG_{2b} previously purified on rProtein A Sepharose Fast Flow.

Column: HiTrap Q (1 mL)
Sample: Purified antibody (0.15 mg) spiked with recombinant Protein A (0.009 mg)
Buffer A: 20 mM Tris-HCl, pH 8.5
Buffer B: 20 mM Tris-HCl, 1.0 M NaCl, pH 8.5
Flow velocity: 300 cm/h
Gradient: 0-25 %B, 20 column volumes

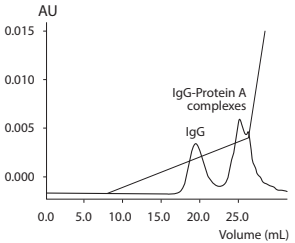

Fig 7. Removal of IgG-Protein A complex from mouse IgG_{2a} by anion exchange chromatography on HiTrap Q HP.
Recombinant Protein A was spiked into IgG_{2a} previously purified on rProtein A Sepharose Fast Flow.

8 Cleaning-In-Place

Cleaning-In-Place (CIP) is used for removal of very tightly bound, precipitated or denatured substances from the resin and the purification system. Accumulation of contaminants may affect the chromatographic properties of the column for example, blocking the column, increasing the back pressure and reducing the flow velocity. Regular CIP prevents the build up of the contaminants in the packed bed, and helps to maintain the capacity, flow properties and general performance of rProtein A Sepharose Fast Flow.

The following CIP protocols should be used as guidelines. Depending on the nature of the contaminants, different protocols need to be used and if the fouling is severe, the protocols may need further optimization. The CIP frequency depends on the nature of the feed material. The recommendation is to use a CIP procedure at least every five cycles of normal use.

CIP procedure

For removal of precipitated or denatured substances follow the procedure below:

Step	Action
1	Wash with two column volumes of 6 M guanidine hydrochloride.
2	Wash immediately with at least 5 column volumes of filter-sterilized binding buffer, pH 7 to 8. ***Note:*** *Use reversed flow direction.*

For removal of hydrophobically bound substances, CIP is performed as follows:

Step	Action
1	Wash the column with two column volumes of a solution containing a non-ionic detergent (e.g., conc. 0.1%).
2	Wash with at least five column volumes of filter-sterilized binding buffer, pH 7 to 8. ***Note:*** *Use reversed flow direction.*

The following is another CIP protocol for removal of hydrophobically bound substances:

Step	Action
1	Wash the column with three to four column volumes of 70% ethanol. ***Note:*** *Use reversed flow direction and an increasing gradient to avoid air bubble formation when using high concentrations of organic solvents.*

CAUTION

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

2	Wash with at least five column volumes of filter-sterilized binding buffer, pH 7 to 8.

9 Sanitization

Sanitization reduces microbial contamination of the bed to a minimum. Follow the steps below to sanitize the column:

Step	Action
1	Equilibrate the column with a solution of 2% hibitane digluconate and 20% ethanol and allow to stand for six hours or Equilibrate the column with a solution of 0.1 M acetic acid and 20% ethanol and allow to stand for one hour.
2	Wash with at least five column volumes of sterile binding buffer.

The following is another sanitization procedure:

Step	Action
1	Equilibrate the column with 70% ethanol.
2	Allow to stand for 12 hours.
3	Wash with at least five column volumes of sterile binding buffer.

CAUTION

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

10 Scaling up

After optimizing the method at laboratory scale, the process can be scaled up. Scale-up to larger columns is typically performed by keeping the bed height and flow velocity (cm/h) constant while increasing the bed diameter and the flow rate.

Scale-up procedure

Step	Action
1	Choose the bed volume according to the required binding capacity.
2	Choose the column diameter to obtain the bed height of 5 to 15 cm so that high flow velocities can be used, see Fig 2. ***Note:*** *Maximum flow velocity is inversely proportional to the bed height. Operate at no more than 70% of the maximum flow velocity.*
3	Define linear flow velocity during sample application to ensure that residence time is not shorter than the established time in the small scale experiments. The residence time is equal to the bed height (cm) divided by the linear flow velocity (cm/h) applied during sample loading.
4	Check the buffer delivery and monitoring systems for time delays or volume changes. ***Note:*** *The use of larger systems when scaling up, may cause some deviations from the optimazied method at the small-scale. For example different lengths and diameters of outlet tubing can cause zone spreading on larger systems.*
5	Keep sample concentration and gradient slope constant.

11 Storage condition

Store unused or packed rProtein A Sepharose Fast Flow as follows:

When	Then
Unused resin	Store the unused resin in its container at 2°C to 8°C, and check that the screw top is fully tightened.
Packed column	Equilibrate the packed column in a buffer containing 20% ethanol to prevent microbial growth.

Note:

Before use, equilibrate with at least five bed volumes of the starting buffer.

12 Ordering information

All bulk resin products are supplied as suspension in 20% ethanol. For additional information, including Data File, Application references and Regulatory Support File, contact your local GE representative.

Product	Quantity	Product code
rProtein A Sepharose Fast Flow	5 mL	17127901
	25 mL	17127902
	200 mL	17127903
	1 L	17127904
	5 L	17127905
	10 L	17127906
	60 L [1]	17127907

[1] Pack sizes available upon request.

For local office contact information, visit
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