cytiva

rmp Protein A
Sepharose Fast Flow

Instructions for Use

rmp Protein A Sepharose™ Fast Flow is a low leakage, non mammalian based affinity resin designed for high purity separation of monoclonal and polyclonal antibodies at laboratory- and process-scales. It has the following characteristics:

- Meets the requirements of modern process design as a result of the very low ligand leakage and the absence of mammalian culture in ligand production and purification
- Increased chemical stability due to mulipoint ligand attachment
- High throughput due to high capacity and good pressure/flow characteristics

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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

rmp Protein A Sepharose Fast Flow is a high capacity, chemically stable BioProcess™ affinity resin designed specially for the purification of monoclonal and polyclonal antibodies at laboratory- and process-scales. 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.

These instructions contain information about resin characteristics, column packing, method design and optimization, scale-up, and maintenance.

2 Characteristics

Recombinant protein A has a molecular weight of M_r 44 600 and contains five antigen binding domains (E, D, A, B, C) which have strong and roughly equal affinity for the Fc region of immunoglobulin. One molecule of protein A can bind approximately two molecules of IgG.

Cytiva recombinant protein A is expressed in *E. coli*, fermented in animal-free soy medium, and is highly purified through multiple step ion exchange chromatography. This manufacturing process is entirely free of contact with human and animal products.

The ligand is immobilized to the base matrix, Sepharose 4 Fast Flow, via reductive amination. This method allows multipoint attachment of the ligand to the matrix and gives increased chemical stability. High chemical stability is an important consideration for cleaning and sanitization procedures which use sodium hydroxide or other basic solutions.

The base matrix Sepharose 4 Fast Flow is a cross-linked 4% agarose with high chemical and physical stabilities. It is particularly suitable for process-scale applications where starting material volumes are large and flow rates are high. The dynamic capacity of rmp Protein A Sepharose 4 Fast Flow is exemplified in *Figure 1, on page 5* where flow velocity/capacity dependence is shown for three dynamic flow velocities. *Figure 2, on page 5* shows a pressure/flow comparison between rmp Protein A Sepharose Fast Flow and two other protein A resins, all manufactured by Cytiva.

Bed dimensions:	0.5 x 4.1 cm bed height
Start buffer:	PBS (pH 7.4)
Sample:	hIgG (Sigma)
Breakthrough capacity:	60 cm/h = 41 mg/mL
	100 cm/h = 32 mg/mL
	200 cm/h = 21 mg/mL

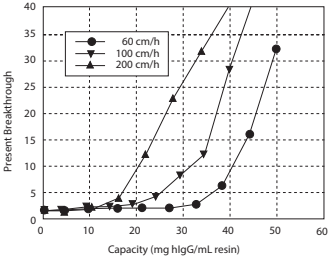


Fig 1. Example of flow velocity/capacity dependence for rmp Protein A Sepharose Fast Flow. Breakthrough capacity for hIgG was determined at three different flow velocities. Breakthrough capacity is defined as mg hIgG applied per mL resin at the point where the concentration of hIgG in the column effluent reaches a value of 10% of the concentration in the sample.

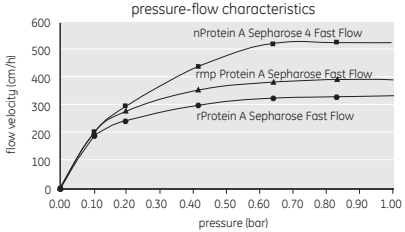


Fig 2. A pressure/flow comparison between three different protein A resins: rmp Protein A Sepharose Fast, rProtein A Sepharose Fast Flow, and nProtein A Sepharose 4 Fast Flow. The pressure/flow data were determined in XK 50/30 (50 mm i.d) column packed to a bed height of 15 cm using 0.1 M NaCl as the mobile phase at 20°C.

Table 1. Characteristics of rmp Protein A Sepharose Fast Flow

Matrix	Cross-linked agarose, 4%, spherical
Particle size, d_{50V} [1]	~ 90 µm
Ligand	Recombinant protein A (*E. coli*)

Coupling chemistry	Reductive amination
Dynamic binding capacity Q_{B10} [2]	≥ 25 mg hIgG/mL resin
Pressure/flow characteristics	150 to 250 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) [3] [4]
Chemical stability	Stable to commonly used aqueous buffers, 20% ethanol 20 mM sodium phosphate 0.1 M glycine/HCl, pH 3.5 0.1 M glycine/HCl, pH 2.5
pH stability, operational [5]	3 to 10 [7]
pH stability, CIP [6]	3 to 12 [7]
Temperature stability	2°C to 40°C
Delivery conditions	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 PEEK 7.5/50 column at 5 cm bed height (3 min residence time) for human IgG in 0.020 M NaH_2PO_4, pH 7

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

4 Pressure/flow test performed on the base matrix

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

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

7 pH below 3 is sometimes required to elute strongly bound IgG species. However, protein ligands can hydrolyze at pH below 2.

3 Packing Columns

rmp Protein A Sepharose Fast Flow is supplied as suspension in 20% ethanol. Decant the 20% ethanol solution and replace it with packing buffer before use.

Recommended columns

Columns	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 to 196 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		

Packing XK columns

Materials needed

- rmp Protein 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. Flow rate 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 procedure

Step	Action
1	Put the packing connector together with another XK column (the second 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 to the column.
3	Put 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.
6	Place the top adapter in the packing tube, sliding it down to the surface of the slurry and displacing the air under the adapter.

Step	Action
7	Pack the column with 20% ethanol at a constant flow rate (see *Table 2, on page 8*, Step 1) and run for 60 min, or until the resin bed is stable.
8	Increase the flow rate (see *Table 2, on page 8*, Step 2), and run for 20 min. ***Note:*** *The packing pressure in step 2 must not exceed 0.15 MPa (1.5 bar, 21.76 psi).*
9	Mark the bed height on the column.
10	Stop the pump, close the column outlet, and dismount the packing tube (if used).
11	Place the adapter in the column tube and adjusted 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 9 with the inlet on top of the column open.

Packing large-scale columns

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.

rmp Protein A Sepharose Fast Flow is easy to pack since their rigidity allows the use of high flow velocities, see *Figure 2, on page 5*. Two suitable types of packing methods are given:

- Chromaflow packing
- Pressure packing (for columns with adapters)

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.

Begin the packing procedure by determining the optimal packing flow rate. Guidelines are given below for determining the optimal packing flow rates for columns with adapters and fixed bed heights.

Determining optimal packing flow rate

The optimal packing flow rate is dependent on column size and type, bed height, packing solution, and temperature. The optimal packing flow rate must therefore 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 bed is approximately 1.15 L sedimented resin.
2	Prepare the column exactly as for column packing.

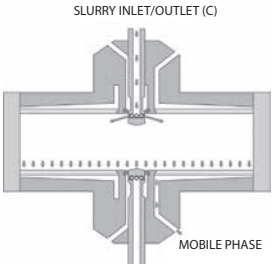
Step	Action
3	Begin packing the column at a low flow rate (e.g., 30% of the expected maximum flow rate) and record the flow rate and back pressure when the bed is packed and the pressure has stabilized.
4	Increase the flow rate in small steps and record the flow rate and pressure at each step after the pressure has stabilized.
5	Continue recording flow rate and pressure until the maximum flow rate has been reached, i.e., when the flow rate levels off at a plateau indicating bed compression or when the pressure reaches the pressure specification 55 of the column used.
6	Plot pressure against flow rate as indicated in *Figure 2, on page 5*. The optimal packing flow rate/pressure is 70% to 100% of the maximum flow rate/pressure.

The operational flow rate/pressure must be < 70% of the packing flow rate/pressure.

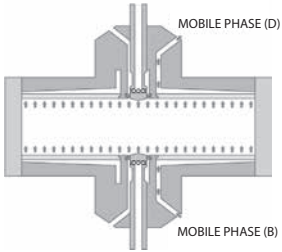
Packing Chromaflow columns

The packing procedure below describes how to pack the the column from the top. However, the column can also be packed from the bottom. To pack from the bottom, do the same procedure for the connections and flow path via the bottom nozzle. Step 1 is the same for both packing methods.

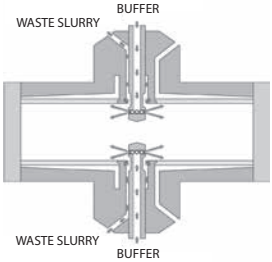
Step	Action
1	Prepare the column for packing as described in the User Manual.
2	Set the top nozzle to the pack position (mid-position).
3	Fully retract the bottom nozzle (run position).
4	Make sure that the top mobile phase is closed.
5	Open the bottom mobile phase.
6	Open Inlet C and start the packing pump. Adjust the flow to achieve the required packing conditions for the selected resin. Monitor column pressure and the outlet flow rate in order to record column packing parameters. (Remember to stir the resin slurry during packing to prevent it from settling.)
7	Continue pumping until the column is fully packed and the pump stalls due to build-up of resin in its pipelines. Turn off the packing pump.
8	Fully retract the top nozzle to its run position. Close Outlet (C). Open Inlet (B) from the water/buffer tank and open Outlet (D). To rinse the top slurry lines, restart the pump. (If the nozzle is full of liquid when in the packing position, make sure that the waste slurry outlet is open before retracting the nozzle.)
9	To clean-in-place, exchange the buffer tank for wash/ buffer tank containing cleaning solution.



Packing position
The top nozzle is extended part of the way (mid position) into the column. The bottom nozzle is fully retracted. Slurry enters the column via the top nozzle and excess liquid exits via the bottom mobile phase outlet. After packing, the slurry lines are isolated from the mobile phase and can be cleaned independently from the rest of the column.



Running position
The bottom and top nozzles are retracted. Mobile phase enters the column directly into an annulus, immediately behind the bed support. The annulus is cut through at an angle to make sure that flow velocity is kept constant during distribution of the mobile phase across the bed.



Unpacking position
In this position, both bottom and top nozzles are fully extended into the column, thereby exposing a third passage through which resin leaves the column.

Cleaning solution can be pumped through the nozzles and sprayed into the column. In this way the column is easily and effectively cleaned without exposing the interior or the resin to the environment, or without dismantling the column.

Fig 3. Principle of operation – Chromaflow columns

Pressure packing (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 a 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 bed support. 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 rate (or pressure). Keep the flow rate (or pressure) constant during packing and check the pressure at the column inlet. Never exceed the pressure limit for column or resin.

Step	Action
5	When the bed has stabilized, close the bottom valve and stop the pump. The bed starts rising in the column. Loosen the O-ring and lower the adapter to 0.5 to 1.0 cm above the bed 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 bed surface and adapter when the bed has stabilized. Mark the bed height on the column tube.
7	Close the bottom valve, stop the pump, 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. 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).

4 Evaluation of packed column

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 deteriorated.

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 depends 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.

Note: *Changing the solute, solvent, eluent, sample volume, flow velocity, liquid pathway, temperature, chromatography system, etc., influence the results.*

For more information about column efficiency testing, consult the application note 28937207.

For optimal column efficiency results, the sample volume must 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 the column use.

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 must be symmetrical, and the asymmetry factor as close to 1 as possible. A typical acceptable range could be $0.8 < A_S < 1.5$.

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.

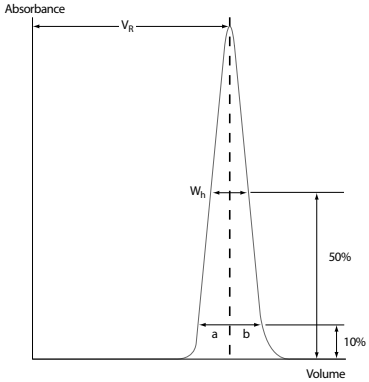


Fig 4. A typical test chromatogram showing the parameters used for HETP and A_S calculations.

5 Method design and optimization

As with most affinity chromatography resins, rmp Protein A Sepharose Fast Flow offers high selectivity which renders efficiency related parameters such as sample load, flow rate, particle size and bed height less important for resolution.

The primary aim of method optimization is to establish the conditions that bind 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 (*Table 3, on page 19*). There might even be a substantial diversity in binding characteristics within a single subclass. This is an important consideration when developing the purification protocol. 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:

- By 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 uninhibited affinity interaction.
- By increasing salt concentration to reduce electrostatic repulsion and to increase hydrophobic interactions.
- By reducing the temperature reported, the dynamic binding capacity will most likely decrease.

Table 3. 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			
IgG1	very high	6.0 to 7.0	3.5 to 4.5
IgG2	very high	6.0 to 7.0	3.5 to 4.5
IgG3	low-none	8.0 to 9.0	≤ 7.0
IgG4	low-high	7.0 to 8.0	3.0 to 6.0
Mouse			
IgG1	low	8.0 to 9.0	4.5 to 6.0
IgG2a	moderate	7.0 to 8.0	3.5 to 5.5

Antibody	Affinity	Binding pH	Elution pH
IgG2b	high	~ 7.0	3.0 to 4.0
IgG3	low-high	~ 7.0	3.5 to 5.5

Method screening

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

An effective way of mapping antibody behaviour on rmp Protein 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. If there is any doubt about this, titrate the antibody fraction to conditions is to reverse the direction of flow during elution. This also elutes the antibody in a more concentrated form.

Recommended screening conditions

Example of suitable buffers:

- *Buffer* A: 20 mM sodium phosphate, 150 mM NaCl, pH 7
- Buffer B: 50 mM sodium phosphate pH 3.0 or 50 mM sodium citrate, pH 3.0

Experimental conditions:

- 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.
- 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.

The linear gradient used in the initial experiment will reveal the relative binding requirements of the target antibody contra the contaminating antibodies.

High salt concentration and high pH will often increase dynamic binding capacity, even for antibodies that bind fairly well to protein A. On the other hand, by decreasing salt concentration and/or pH during binding, it is possible to avoid binding contaminating antibodies. This can also increase dynamic binding capacity since more binding sites will be available for the target antibody. It will also increase selectivity in the system. The balance between selectivity and capacity must be defined with respect to the nature of the feed, that is, presence of contaminating antibodies and the purity requirement in the eluted product.

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

When optimizing elution conditions, determine the highest pH that allows efficient desorption of antibody from the column. This will prevent denaturation of sensitive antibodies due to exposure to low pH values. Stepwise elution (*Figure 5, on page 23*) is often prefered in large-scale applications since it is technically simpler than elution with continuous gradients. It also allows the target monoclonal antibody to be eluted in a more concentrated form and provides decreased buffer consumption and shorter cycle times. Linear gradient elution can be feasible for scale-up. Its main advantage is that it provides the best and most reproducible fractionation from contaminating antibodies.

Column:	rmp Protein A Sepharose Fast Flow, HR 10/10, bed height 7.0 cm (5.5 mL)
Sample:	1 mg/mL hIgG, Gammanorm™
Sample volume:	55 mL containing 55 mg hIgG
Starting buffer:	20 mM PBS in 0.15 M NaCl, pH 7.4
Elution buffer:	0.1 M sodium citrate, pH 3.0
Flow velocity:	140 cm/h (= 1.83 mL/min) at elution 47 cm/h (= 0.62 mL/min)

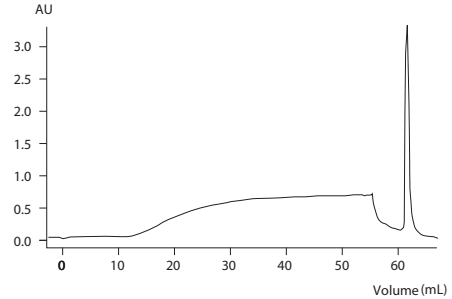


Fig 5. Frontal analysis on rmp Protein A Sepharose Fast Flow using human IgG, followed by step elution.

Optimization of throughput

When optimizing for highest throughput and productivity it is necessary to define the highest sample load over the shortest sample application time with the most acceptable loss in product recovery. The dynamic binding capacity for the target antibody must be determined by frontal analysis using real process feedstock. Since the dynamic binding capacity is a function of the flow velocity applied during sample application, the breakthrough capacity must be defined over a range of different flow rates. The optimal flow rate is that which gives the highest throughput in terms of amount of antibody processed per time unit and volume of resin. Example of breakthrough profiles at different flow rates are shown in *Figure 1, on page 5*.

Removal of leached protein A from final product

Leakage of protein A from rmp Protein A Sepharose Fast Flow is generally low. However, in many monoclonal applications it is a requirement that leached protein A is eliminated from the final product. There are numerous chromatographic solutions to this problem. Below are suggestions for subsequent chromatographic steps:

- Size exclusion chromatography can be applied for removal of protein A-IgG aggregates by conducting the separation under moderate pH conditions. The large IgG-protein A complexes that are formed will elute early from the column (*Figure 6, on page 25*).
- Cation exchange chromatography is an effective tool for removing residual protein A, 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 (*Figure 7, on page 26*).
- Anion exchange chromatography can also be used to reduce leached protein A contamination. 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 noncomplex antibodies (*Figure 8, on page 27*). These 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, equilibrate the column to 20 mM Tris-HCl, pH 8.5, apply sample and 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™ 200 pg, 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

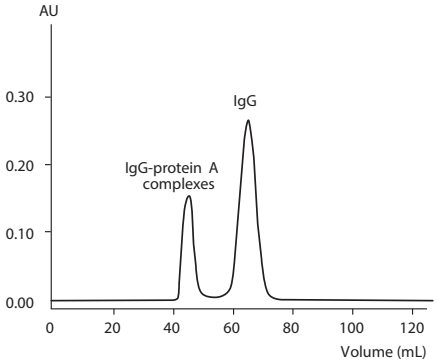


Fig 6. Removal of IgGprotein A complex from mouse IgG_{2a} by size exclusion chromatography on Superdex 200 prep grade. Recombinant protein A was spiked into mouse IgG_{2a}.

Column:	HiTrap™ SP HP (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% to 45%B, 15 column volumes

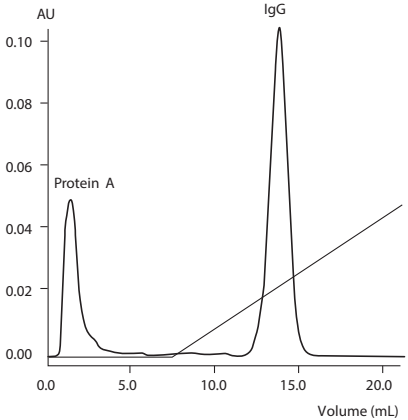


Fig 7. Removal of protein A from mouse IgG_{2b} by cation exchange chromatography on HiTrap SP HP. Recombinant protein A was spiked into mouse IgG_{2b}.

Column:	HiTrap Q HP (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

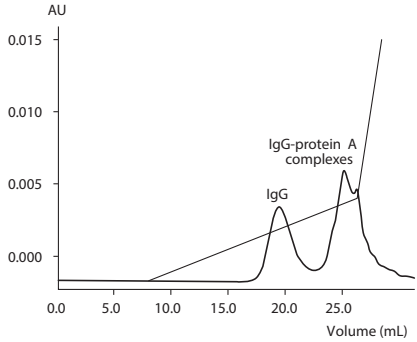


Fig 8. Removal of IgG-protein A complex from mouse IgG_{2a} by anion exchange chromatography on HiTrap Q HP. Recombinant protein A was spiked into IgG_{2a}.

6 Cleaning-in-place (CIP)

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 can affect the chromatographic properties of the column. If the fouling is severe, it can block the column, increase back pressure, and reduce flow rate.

Regular CIP prevents the buildup of these contaminants in the packed bed, and helps to maintain the capacity, flow properties, and general performance of rmp Protein A Sepharose Fast Flow.

The general recommendation for cleaning rmp Protein A Sepharose Fast Flow is to use sodium hydroxide at a concentration of 10 to 50 mM. As an alternative cleaning protocol 6 M guanidine hydrochloride can be used. A contact time of a minimum of 10 minutes is recommended for the CIP-solutions. To remove hydrophobically bound substances, a solution of nonionic detergent or ethanol is recommended.

The CIP protocols should be used as guidelines for formulating a cleaning protocol specific for the feed material applied to the column. The frequency of use will depend on the nature of the feed material but it is recommended to use a CIP procedure at least every 5 cycles during normal use. Depending on the nature of the contaminants, different protocols maybe have to be used in combination. If fouling is severe, the protocols maybe have to be further optimized.

7 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.

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

8 Storage

Unused resin can be stored in the container at a temperature of 2°C to 8°C in 20% ethanol. Make sure that the screw top is fully tightened.

Packed columns must be equilibrated in binding buffer containing 20% ethanol to prevent microbial growth.

After storage, equilibrate with at least 5 bed volumes of starting buffer before use.

9 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.

- Select bed volume according to required binding capacity.

- Select column diameter to obtain a bed height of 5 to 15 cm so that high flow rates can be used. (See *Figure 2, on page 5*, pressure/flow curve. Maximum flow rate is approximately inversely proportional to the bed height. Expect to operate at no more then 70% of the maximum flow rate.)
- Define flow velocity during sample application to make sure that residence time is not shorter than that established in the small-scale experiments. The residence time is equal to the bed height (cm) divided by the flow velocity (cm/h) applied during sample loading.
- Keep sample concentration and gradient slope constant.

The larger equipment needed when scaling up can 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.

10 Ordering information

Product	Quantity	Product code
rmp Protein A Sepharose Fast Flow	5 mL	17513801
	25 mL	17513802
	200 mL	17513803
	1 L	17513804
	5 L	17513805
Lab-scale columns		
XK 16/20 (16 mm i.d.)		18877301
XK 26/20 (26 mm i.d.)		18100072

Product	Quantity	Product code
Data Files		
Chromaflow columns		18113892
BGP columns		18111523

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

11 Further information

For the latest news, more product information and our handbooks, visit: *cytiva.com/protein-purification*

cytiva.com

For technical support, visit: *cytiva.com/techsupport*

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

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