Protein G Sepharose™ 4 Fast Flow

Protein G Sepharose 4 Fast Flow is an affinity resin with protein G immobilized to Sepharose 4 Fast Flow by the CNBr method.

Protein G binds to the Fc region of IgG from a variety of mammalian species. Protein G Sepharose 4 Fast Flow can be used to isolate and purify classes, subclasses and fragments of immunoglobulins from any biological fluid or cell culture medium. Protein G Sepharose 4 Fast Flow is extremely useful for isolation of immune complexes. See *Section 5 Immunoprecipitation, on page 11* for detailed instructions on immunoprecipitation application.

The potential applications of protein G include practically all of the current and projected applications of protein A. Protein G and protein A, however, have different IgG binding specificities, dependent on the origin of the IgG. Compared to protein A, protein G binds more strongly to polyclonal IgG, for example, from cow, sheep and horse. Furthermore, unlike protein A, protein G binds polyclonal rat IgG, human IgG3 and mouse IgG1.

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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™ resins

BioProcess 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.

2 Product description

Recombinant protein G, M_r 17 000, from GE is produced in *E. coli* and contains two IgG binding regions. The albumin binding region of native protein G has been genetically deleted, thereby avoiding undesirable cross-reactions with albumin. The pI of protein G is 4.4 and the pH stability 2 to 10.

Table 1. Characteristics of Protein G Sepharose 4 Fast Flow

Matrix	Cross-linked agarose, 4%, spherical
Particle size, d_{50v} [1]	~ 90 µm
Ligand concentration	~ 2 mg Protein G/mL resin
Pressure/flow characteristics	150-250 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 [2], [3])
Total binding capacity [4]	≥ 20 mg human IgG/mL resin
pH stability, operational [5]	3 to 9 [7]
pH stability, CIP [6]	2 to 10 [7]
Chemical stability	Stable to commonly used aqueous buffers, 6 M guanidine hydrochloride (pH 4.7), 20 mM sodium phosphate with 1% SDS (pH 7), 70% ethanol, 20 mM sodium phosphate, 100 mM glycine-phosphoric acid, 6 M urea, 20% ethanol with 2% hibitane digluconate
Physical stability	Negligible volume variation due to changes in pH or ionic strength.
Sanitization	Sanitize the column with 70% ethanol
Storage	20% ethanol, 2°C to 8°C

1 Median particle size of the cumulative volume distribution.

2 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.

3 Pressure/flow test performed on the base matrix.

4 Protein in excess is loaded in 0.020 M NaH_2PO_4 at pH 7 on a HR 10/19 column. The binding capacity is obtained by measuring the amount of eluted protein in 0.1 M Glycine at pH 3.

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 may hydrolyze at pH below 2.

Table 2. Relative binding strengths for protein A and protein G

Species	Subclass	Protein A binding [1]	Protein G binding
Human	IgA	variable	-
	IgD	-	-
	IgE	-	-
	IgG_1	++++	++++
	IgG_2	++++	++++
	IgG_3	-	++++
	IgG_4	++++	++++
	IgM	variable	-
Avian egg yolk	IgY	-	-
Cow		++	++++
Dog		++	+
Goat		-	++
Guinea pig	IgG_1	++++	++
Hamster		+	++
Horse		++	++++
Koala		-	+
Llama		-	+
Monkey (rhesus)		++++	++++
Mouse [2]	IgG_1	+	++++
	IgG_{2a}	++++	++++
	IgG_{2b}	+++	+++
	IgG_3	++	+++
	IgM	variable	-
Pig		+++	+++
Rabbit	No distinction	++++	+++
Rat [3]	IgG_1	-	+
	IgG_{2a}	-	++++
	IgG_{2b}	-	++
	IgG_3	+	++
Sheep		+/-	++

[1] ++++ = strong binding; ++ = medium binding; - = weak or no binding

[2] IgG_1 from mouse binds more strongly to protein G than to protein A.

[3] Note that IgG from rat binds to protein G coupled to Sepharose 4 Fast Flow.

3 Operation

Preparing the resin

Let the resin settle overnight in a measuring cylinder. Prepare a 45% to 55% slurry by adding 20% ethanol to the settled resin.

Packing of Protein G Sepharose 4 Fast Flow

Materials needed

- Protein G Sepharose 4 Fast Flow
- XK column (16, 26, or 50)

  XK packing connector XK16 or XK26
- HiScale™ column (16, 26, or 50)

  HiScale 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 3. 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	Attach 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 mount 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 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	Attach 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 3, Step 1) and run for 60 min or until the resin bed is stable.
8	Increase the flow (Table 3, Step 2) and run for 20 minutes. ***Note:*** *The packing pressure in step 2 should not exceed 1.5 bar.* ***Note:*** *Do not exceed 75% of the packing flow rate in subsequent purification procedures.*
9	Mark the bed height on the column.
10	Stop the pump, close the column outlet and dismount the packing tube (if used).

Step	Action
11	Assemble 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 10 with the inlet on top of the column open.

Binding

IgG from most species binds Protein G Sepharose 4 Fast Flow at neutral pH and physiological ionic strength.

As a general method we recommend 20 mM sodium phosphate, pH 7.0 as binding buffer.

The binding capacity of Protein G Sepharose 4 Fast Flow depends on the source of the particular immunoglobulin, see *Table 4*.

However, the total capacity depends upon several factors, such as the flow rate during sample application, the sample concentration and binding buffer. The table below shows the total capacity under defined conditions for IgG from some species.

Table 4. The table below shows a comparison of the total capacity under defined conditions [1] for IgG from some species.

Species	Total IgG capacity
Human	17
Rat	7
Sheep	18
Rabbit	19
Goat	19
Guinea-pig	17
Cow	23
Mouse [2]	6

[1] The total capacity was determined with 1 mL drained resin packed in a 1 × 10 cm column at a flow velocity of approx. 11 cm/h. Approximately 40 mg IgG was applied. Binding buffer used was 20 mM sodium phosphate, pH 7.0 and elution buffer used was 0.1 M glycine-HCl, pH 2.7.

[2] Extrapolated value from experiment carried out at 1/5th scale.

Note:
The binding capacity values listed above are typical for the given species. However, there might be considerable deviations in binding capacity for different immuno-globulins derived from the same species, even if they are of the same subclass.

Elution

To elute IgG from Protein G Sepharose 4 Fast Flow it is necessary to lower the pH to about 3.0 to 2.5 depending on the IgG.

As a general method, we recommend 0.1 M glycine buffer, pH 3.0 to 2.5 as elution buffer.

As a safety measure to preserve the activity of acid labile IgG's, it is recommended to add 60 to 200 µL/mL eluate of 1 M Tris-HCl, pH 9.0, to neutralize the eluted fractions.

Regeneration

After elution, the resin should immediately be washed with 2 to 3 bed volumes of elution buffer followed by re-equilibration with 2 to 3 bed volumes of binding buffer.

In some applications, substances like denatured proteins or lipids, do not elute in the regeneration procedure. These can be removed by cleaning-in-place procedures.

4 Cleaning-in-place and Sanitization

Cleaning-in-place (CIP)

Remove strongly bound hydrophobic proteins, lipoproteins and lipids by washing the column with a nonionic detergent, 0.1%, at 37°C, contact time one minute. Immediately re-equilibrate with at least 5 bed volumes of sterile filtered binding buffer.

Alternatively, wash the column with 70% ethanol and let stand for 12 hours. Re-equilibrate with at least 5 bed volumes of sterile binding buffer.

Sanitization

Sanitization reduces microbial contamination of the chromatography resin to a minimum.

Wash the column with a buffer containing 2% hibitane digluconate and 20% ethanol. Allow to stand for 6 hours.

Re-equilibrate the column with 3 to 5 bed volumes of sterile binding buffer.

Column performance is normally not significantly changed by the cleaning in place or sanitization procedures described above.

These recommended cleaning procedures can be performed directly on the packed column.

5 Immunoprecipitation

Immunoprecipitation is a highly specific and effective technique for analytical separations of target antigens from crude cell lysates.

Getting started

To obtain satisfactory results using immunoprecipitation, all procedures involved must be empirically optimized. For example, selecting cell lysis conditions is very critical and has to be optimized with regard to cell type and how the antigen is to be used. Whereas cells without cell walls (e.g., animal cells) are easily disrupted by treatment with mild detergent, other cells might need some type of mechanical shearing such as sonication or homogenization.

The parameters listed below (lysis buffers, incubation times, volumes, and concentrations) should therefore be regarded as guidelines for initial experiments.

For common problems and general tips, see instructions *Immunoprecipitation Starter Pack* (71501754).

Preparing the resin

Protein G Sepharose 4 Fast Flow is supplied preswollen in 20% ethanol. Wash the resin three times with lysis buffer. Centrifuge at 12 000 g for 20 seconds between the washes and discard the supernatant . Prepare a 50% slurry by mixing equal volumes of resin and lysis buffer. Store at 4ºC and mix well before use.

Cell lysis

Step	Action
1	• **Adherent cells:** Remove all culture medium and wash twice with ice-cold PBS. Discard the supernatants and drain well. • **Cells in suspension:** Collect cells by centrifugation at 1000 g for 5 minutes and discard the culture medium supernatant. Resuspend the pellet in ice-cold PBS, centrifuge and discard the supernatant . Repeat the wash once.
2	• **Adherent cells:** Place the tissue culture dish on ice. Add ice-cold lysis buffer[1] to a concentration of 10^6 to 10^7 cells/mL (1 mL to a cell culture plate, Ø 10 cm). Incubate on ice for 10 to 15 minutes with occasional rocking. • **Cells in suspension:** Suspend the washed pellet in ice-cold lysis buffer[1] at a concentration of 10^6 to 10^7 cells/mL (approximately 10 cell volumes lysis buffer). Incubate on ice for 10 to 15 minutes with gentle mixing.
3	Transfer the cells to a suitable homogenization tube.
4	Further disrupt the cells by sonication, homogenization or passage through a 21 Gauge needle. Keep the cells on an ice bath to prevent the temperature from rising.
5	Centrifuge at 12 000 g for 10 minutes at 4°C to remove particulate matter.
6	Transfer the lysate (the supernatant) to a fresh tube. Keep on ice.

[1] See Section *Buffers and solutions* for help when selecting lysis buffer.

Preclearing (optional)

Antibodies present in the cell lysate might also bind to the resin and thus interfere with subsequent analysis. In such a case preclearing might be desired.

Step	Action
1	Add 50 to 100 µL Protein G Sepharose 4 Fast Flow suspension (50% slurry) to 1 mL cell lysate in an Eppendorf™ tube. Higher volume of resin might be necessary when working with serum samples due to the large amount of IgG present.
2	Gently mix for 1 hour at 4°C.
3	Centrifuge at 12 000 g for 20 seconds. Save the supernatant.

Couple antigen to antibody

Step	Action
1	Aliquot samples (500 µL) in new Eppendorf tubes.
2	Add • polyclonal serum (0.5 to 5 µL), • hybridoma tissue culture supernatant (5 to 100 µL), • ascites fluid (0.1 to1 µL), or purified monoclonal or polyclonal antibodies (add the volume corresponding to 1 to 5 µg). For controls, use nonimmune antibodies that are as close to the specific antibody as possible (for example, polyclonal serum should be compared to normal serum from the same species).
3	Gently mix for 1 hour at 4°C.

Precipitation of the immune complexes

Step	Action
1	Add 50 µL Protein G Sepharose 4 Fast Flow suspension (50% slurry). ***Note:*** *It is possible to work with volumes down to 10 µL.*
2	Gently mix for 1 hour at 4°C.
3	Centrifuge at 12 000 g for 20 seconds and save the pellet.
4	Wash the pellet three times with 1 mL lysis buffer and once with wash buffer. Centrifuge at 12 000 g for 20 seconds between each wash and discard the supernatants. ***Note:*** *Be very careful when removing the supernatants to avoid loss of the beads!*

Dissociation and analysis

Step	Action
1	Suspend the final pellet in 30 µL sample buffer.
2	Heat to 95°C for 3 minutes.
3	Centrifuge at 12 000 g for 20 seconds to remove the beads. Carefully remove the supernatant.
4	Add 1 µL 0.1% bromphenol blue.
5	Analyze the supernatant by SDS-PAGE, followed by protein staining and/or immunoblotting for detection. Radiolabeled antigens are detected by autoradiography.

Buffers and solutions

Lysis buffers

Cell lysis must be harsh enough to release the target antigen, but mild enough to maintain its immunoreactivity. Selecting lysing conditions is therefore very critical and has to be individually optimized.

Some commonly used lysis buffers are listed below. NP-40 (IGEPAL CA-630) and RIPA buffer release most soluble cytoplasmic or nuclear proteins without releasing chromosomal DNA and are a good choice for initial experiments. Some parameters that affect the extraction of an antigen include salt concentration (0 to 1 M), nonionic detergents (0.1% to 2%), ionic detergents (0.01% to 0.5% and pH (6 to 9).

Name	Description	Strin-gency
Low salt	1% IGEPAL CA-630, 50 mM Tris, pH 8.0, 1 mM PMSF	+
Mammalian Protein Extraction Buffer	Tris-based buffer, 10 mM NaCl, nonionic detergent mixture, pH 7.5	+
Yeast Protein Extraction Buffer	Tris-based buffer, 50 mM NaCl, nonionic detergent mixture, pH 7.5	+
NP-40 (IGEPAL CA-630)	150 mM NaCl, 1% IGEPAL CA-630, 50 mM Tris, pH 8.0, 1 mM PMSF	++
RIPA	150 mM NaCl, 1% IGEPAL CA-630, 0.5% sodium deoxycholate (DOC), 0.1% SDS, 50 mM Tris, pH 8.0, 1 mM PMSF	+++
High salt	500 mM NaCl, 1% IGEPAL CA-630, 50 mM Tris, pH 8.0, 1 mM PMSF	++++

Other buffers/solutions

Name	Description
PBS	1 mM KH_2PO_4, 10 mM Na_2HPO_4, 137 mM NaCl, 2.7 mM KCl, pH 7.4
Wash buffer	50 mM Tris, pH 8
Sample buffer (reducing)	1% SDS, 100 mM DTT, 50 mM, Tris, pH 7.5

6 Storage

For storage, keep the resin at 2°C to 8°C in a suitable bacteriostat, e.g., 20% ethanol. Protein G Sepharose 4 Fast Flow must not be frozen.

7 Ordering information

Product	Quantity	Product code
Protein G Sepharose 4 Fast Flow [1]	5 mL	17061801
	25 mL	17061802
	200 mL	17061805
	1 L	17061806
	5 L	17061804

[1] Larger quantities are available. Contact GE for more information.

Related products	Quantity	Product code
HiTrap™ Protein G HP	5 × 1 mL	17040401
	2 × 1 mL	17040403
	1 × 1 mL	29048581
	1 × 5 mL	17040501
	5 × 5 mL	17040503
MAbTrap™ Kit	1 kit	17112801
Ab SpinTrap™	50 × 100 µL	28408347
Immunoprecipitation Starter Pack nProtein A Sepharose 4 Fast Flow, 2 mL Protein G Sepharose 4 Fast Flow, 2 mL	2 × 2 mL	17600235
Protein G HP SpinTrap	16 × 100 µL	28903134
Protein G HP MultiTrap™	4 × 96-well plates	28903135
Ab Buffer Kit	1 kit	28903059
Protein G	5 mg	17061901
Protein A Mag Sepharose	500 µL	28944006
	4 × 500 µL	28951378
Protein G Mag Sepharose	500 µL	28944008
	4 × 500 µL	28951379

Product	Quantity	Product code
Antibody Purification Handbook	1	18103746
Solutions for antibody purification, Selections Guide	1	28935197
Affinity Chromatography Handbook	1	18102229
Prepacked chromatography columns for ÄKTA systems, Selection guide	1	28931778

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