Protein G Sepharose 4 Fast Flow

Protein G Sepharose™ 4 Fast Flow is recombinant protein G coupled to Sepharose 4 Fast Flow.

Protein G Sepharose 4 Fast Flow offers:

- Broad IgG binding spectrum
- Binding specificity of recombinant Protein G ligand complements the different Protein A Sepharose media available from GE Healthcare
- No specific albumin binding
- Optimized homogeneous recombinant ligand
- High capacity

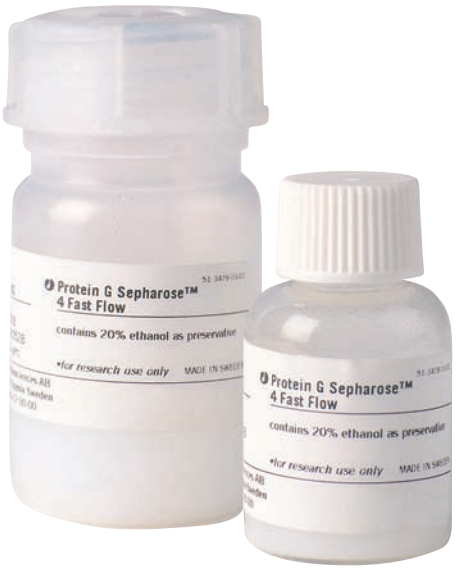


Fig 1. Protein G Sepharose Fast Flow 5 ml and 25 ml.

Medium characteristics

Protein G is immobilized by the well documented CNBr method on Sepharose 4 Fast Flow, a highly cross-linked 4% agarose derivative with unique chemical and physical stability. The kinetics of the matrix impart excellent chromatographic properties to the affinity adsorbent, which ensures high yields of separated IgG (Table 1).

The characteristics of Protein G Sepharose 4 Fast Flow are summarized in Table 2.

Binding capacity

The binding capacity of Protein G Sepharose 4 Fast Flow for IgG depends upon the source species of the particular immunoglobulin. The dynamic capacity depends upon several factors, such as flow rate during sample application, and sample concentration. Table 1 shows the dynamic capacity for IgG from some species under defined conditions. The capacity for human IgG given in Table 1 differs from the value in Table 2 as the latter value was obtained by applying a sample of IgG of the same volume, but different concentration.

Table 1. The dynamic IgG capacity of Protein G Sepharose 4 Fast Flow for various species (evaluation performed at GE Healthcare[1])

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

[1] Conditions used to determine dynamic capacity were as follows:

Medium	Protein G Sepharose 4 Fast Flow
Sample	50-mg pure polyclonal IgG in 10-ml binding buffer
Bed dimensions	0.5 × 5 cm, VT: 1 ml
Flow rate	0.15 ml/min (45 cm/h)
Binding buffer	20 mM sodium phosphate, pH 7.0
Elution buffer	100 mM glycine-HCl, pH 2.7

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

Stability

Protein G Sepharose 4 Fast Flow maintains the IgG binding capacity and recovery after storage in all commonly used aqueous buffers and denaturants such as 6 M guanidine hydrochloride and 8 M urea, and chaotropic salts such as 3 M sodium isothiocyanate.

The medium cannot be autoclaved but may be sanitized by washing with 70% ethanol.

Operation and regeneration

Protein G Sepharose 4 Fast Flow is supplied pre-swollen in 20% ethanol.

To pack the medium, first wash away the ethanol solution with distilled water (pH 7) on a sintered glass filter or similar. While the medium is still on the filter, resuspend it in binding buffer, for example 20 mM phosphate buffer, pH 7.0, and transfer it to the column. Pack the column.

Equilibrate the column with two column volumes of binding buffer, and the column is ready for use. Complete packing instructions are supplied.

To prolong the working life of the medium, samples should be pretreated, for example, centrifugation followed by filtration through a 0.22 µm filter. We recommend that the pH of the sample should be the same as that of the binding buffer. If it is not, adjust the pH of the sample with binding buffer.

Protein G Sepharose 4 Fast Flow binds IgG over a wide pH range, accommodating a wide variety of buffers, depending on the application.

Table 2. Characteristics of Protein G Sepharose 4 Fast Flow

Ligand	Recombinant Streptococcal protein G lacking the albumin-binding region, produced in *E. coli*
Number of IgG binding sites per ligand	2
Molecular weight (M_r) of ligand	Approximately 17 000
pI of ligand	4.1
Ligand density	Approximately 2 mg protein G/ml drained medium
Ligand coupling method	Cyanogen bromide activation
Dynamic binding capacity[1]	Approximately 18 mg human IgG/ml drained medium
Matrix	Spherical, highly cross-linked agarose, 4%
Average particle size	90 µm (45–165 µm)
Chemical stability	IgG binding capacity and recovery was maintained after storage for: *7 d at 37°C in:* 1 M acetic acid, pH 2.0; 20 mM sodium phosphate, 1% SDS, pH 7.0; 6 M guanidine-HCl, pH 7.0; 70% ethanol *2 h at room temperature in:* 0.1 M HCl, pH 1.0; 8 M urea, pH 10.5; 0.1 M glycine-NaOH, pH 11
pH stability[2]	
Long term	3–9
Short term	2–10
Recommended flow rate	50-300 cm/h
Max operating back pressure	0.1 MPa (1 bar, 14 psi)
Sanitization	Wash the packed column with 70% ethanol
Storage	20% ethanol at 4°C to 8°C

[1] Dynamic binding capacity calculated according to the quantity of protein bound at a point on the rising breakthrough curve where 1% of the absorbance signal of the protein sample is reached. The capacity was determined at a linear flow rate of 30 cm/h and with a sample concentration of 0.92 mg/ml. Please note that there may be considerable deviation in binding capacity for different immunoglobulins derived from the same species, even if they are of the same subclass.

[2] Complete data on the stability of protein G as a function of pH are not available. The ranges given are estimates based on our knowledge and experience. Please note the following:
pH stability, long term refers to the pH interval where the medium is stable over a long period of time without adverse effects on its subsequent chromatographic performance.
pH stability, short term refers to the pH interval for regeneration, cleaning-in-place, and sanitization procedures.
pH below 3 is sometimes required to elute strongly bound IgG species. However, protein ligands may hydrolyze at very low pH.

In choosing a flow rate for washing away unbound material and for subsequent elution of bound IgG from the column, 60 cm/h is a good starting point.

The binding between the immobilized protein G and the IgG is strong. To elute the IgG, it may be necessary to lower the pH to below 3.0, depending on the sample origin. As a security measure to preserve the activity of acid labile IgG, we recommend adding 60 to 200 µl of 1 M Tris™-HCl, pH 9.0 per ml fraction (determine the exact amount experimentally), to those tubes destined to collect the fractions containing IgG, so that the final pH of the fraction will be approximately neutral.

After elution, the column should be re-equilibrated with binding buffer. For longer periods of storage, the recommended storage conditions are 20% ethanol at 4°C to 8°C (to prevent microbial contamination).

Applications

Protein G binds specifically to the Fc portion of IgG from most mammalian species. Some of the most important application areas for Protein G Sepharose 4 Fast Flow are the isolation and purification or the removal of IgG from serum, the purification of monoclonal antibodies, and the isolation of immune complexes. Since, in many cases, IgG binds more strongly to Protein G Sephrose 4 Fast Flow than to different kinds of Protein A Sepharose media, the former medium becomes a valuable tool to increase yield or to use in cases where protein A shows little or no interaction.

Purification of polyclonal IgG from serum

Purification of IgG from serum can be carried out simply and effectively in a single step with Protein G Sepharose 4 Fast Flow. IgG from human, cow (Fig 2), horse, sheep, guinea-pig, dog, rabbit, mouse, and rat have all been successfully purified in our laboratories. Protein G Sepharose 4 Fast Flow can also be used for the purification of IgG from many other species (see Table 3).

Medium: Protein G Sepharose 4 Fast Flow
Sample: 100 µl bovine serum, centrifuged and filtered (0.22 µm)
Bed dimensions: 1 × 1.2 cm, V_t: 0.94 ml
Flow rate: 0.8 ml/min (61 cm/h)
Binding buffer (A): 20 mM sodium phosphate, pH 7.0
Elution buffer (B): 0.1 M glycine-HCl, pH 2.7

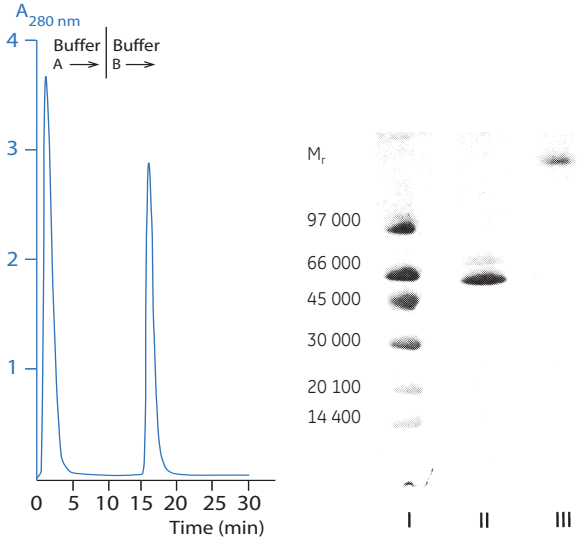


Fig 2. Protein G Sepharose 4 Fast Flow is an efficient medium for simple, rapid and high-yield one-step separation of IgG from serum. SDS-PAGE of the non-reduced samples was run on PhastSystem™ using PhastGel™ 8–25 and Coomassie™ blue staining. Lane I, Low Molecular Weight Calibration Kit (GE Healthcare); Lane II, unbound fraction; Lane III, purified IgG fraction (40 µg sample).

Purification of monoclonal antibodies

Because of its binding characteristics, Protein G Sepharose 4 Fast Flow is a valuable tool for the one-step separation of monoclonal antibodies (MAbs) from ascites and cell culture fluid as well as recombinant antibodies. In fact, the Sepharose 4 Fast Flow matrix makes this medium particularly suitable for separations from cell culture fluid, where it is often necessary to rapidly process large volumes.

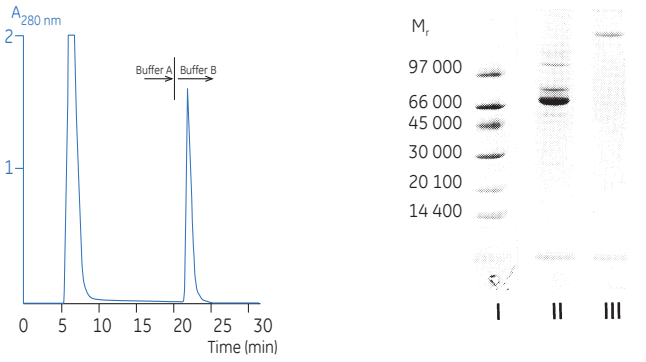
Table 3. The relative binding strengths of antibodies from various species to protein A and protein G as measured in a competitive ELISA test. The amount of IgG required to give a 50% inhibition of binding of rabbit IgG conjugated with alkaline phosphatase was determined.

Species	Subclass	Protein A binding	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	++++	++
	IgG_2	++++	++
Hamster		+	++
Horse		++	++++
Koala		—	+
Llama		—	+
Monkey (rhesus)		++++	++++
Mouse	IgG_1	+	++++
	IgG_{2a}	++++	++++
	IgG_{2b}	+++	+++
	IgG_3	++	+++
	IgM*	variable	—
Pig		+++	+++
Rabbit		++++	+++
Rat	IgG_1	—	+
	IgG_{2a}	—	++++
	IgG_{2b}	—	++
	IgG_3	+	++
Sheep		+/—	++

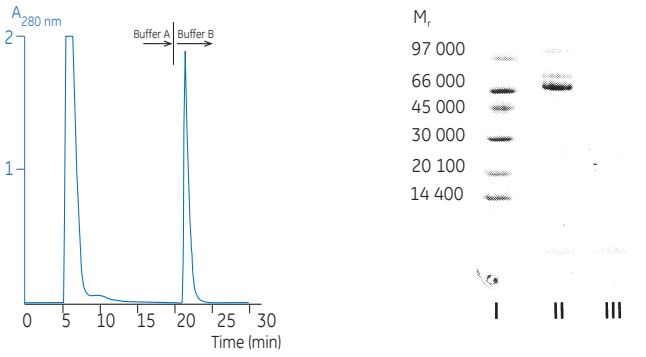
* Purified using HiTrap™ IgM Purification HP columns, see related products
† Purified using HiTrap IgY Purification HP columns, see related products
++++ = strong binding
++ = medium binding
— = weak or no binding

Medium: Protein G Sepharose 4 Fast Flow
Sample: 100 µl mouse ascites in 100 µl binding buffer, centrifuged
Bed dimensions: 1 × 1.2 cm, V_t: 0.94 ml
Flow rate: 0.8 ml/min (61 cm/h)
Binding buffer (A): 20 mM sodium phosphate, pH 7.0
Elution buffer (B): 0.1 M glycine-HCl, pH 2.7

a. Mouse IgG_1



b. Mouse IgG_{2a}



c. Mouse IgG_{2b}

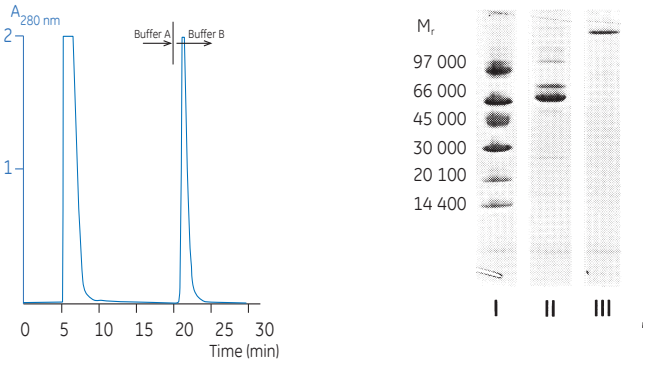


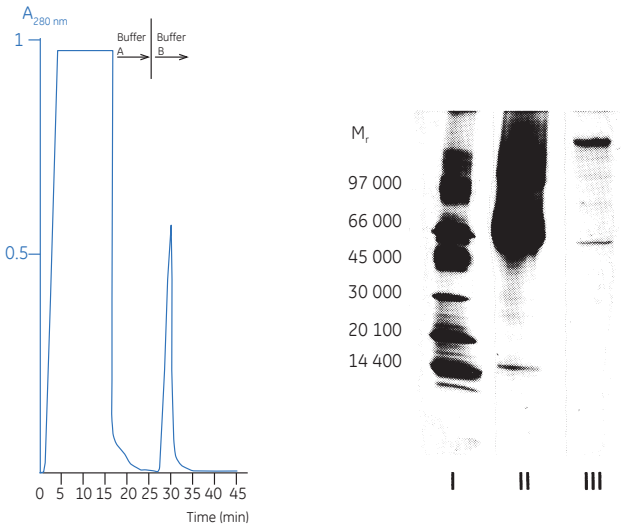
Fig 3. Protein G Sepharose 4 Fast Flow is used for the separations of monoclonal IgG from mouse ascites. The Figure shows the separation of three different subclasses of mouse IgG: (a) IgG_1 (anti-CEA); (b) IgG_{2a} (anti-Apo A); (c) IgG_{2b} (anti-insulin). Adjacent insets show the SDS-PAGE of corresponding non-reduced samples. Samples were run on PhastSystem using PhastGel 8–25 and Coomassie blue staining. Lane I, Low Molecular Weight Calibration Kit (GE Healthcare); Lane II, unbound fraction; Lane III, purified MAb fraction.

Note: *With Protein G Sepharose 4 Fast Flow, the sample can be applied to the column under physiological conditions, thus avoiding high salt concentrations in the binding buffer (see Fig 3 and Fig 4).*

In our laboratories, all tested MAbs of different subclasses from mouse and rat have been separated successfully with a typical recovery of 70% to 90% of the original activity, as measured by ELISA. With the natural diversity of MAbs, however, it is likely that some will bind only weakly or not at all.

Medium: Protein G Sepharose 4 Fast Flow
Sample: 10 µl cell culture fluid, filtered (0.22 µm)
Bed dimensions: 1 × 1.2 cm, V_t: 0.94 ml
Flow rate: 0.8 ml/min (61 cm/h)
Binding buffer (A): 20 mM sodium phosphate, pH 7.0
Elution buffer (B): 0.1 M glycine-HCl, pH 2.7

a. Rat IgG_{2a}



b. Rat IgG_{2b}

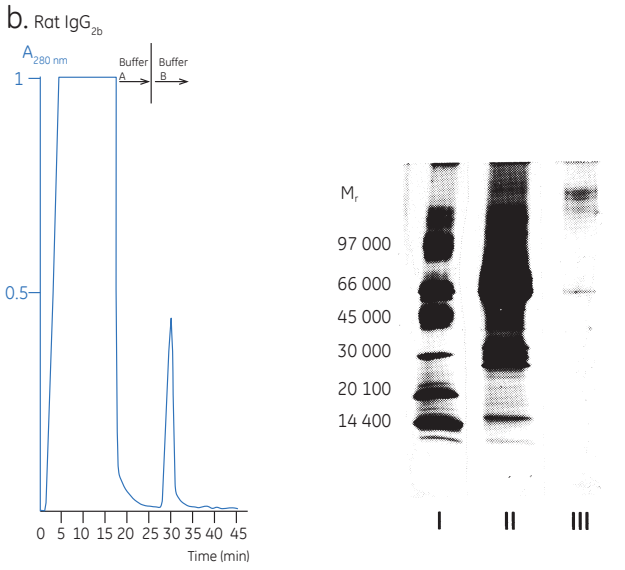


Fig 4. Purification of monoclonal IgG from rat hybridoma cell culture fluid. The figure shows the purification of two different subclasses of rat IgG: (a) IgG_{2a} (anti-CD4); (b) IgG_{2b} (anti-CD4). Adjacent insets show the SDS-PAGE of corresponding non-reduced samples. Samples were run on PhastSystem using PhastGel 8–25 and silver staining. Lane I, Low Molecular Weight Calibration Kit (GE Healthcare); Lane II, unbound fraction; Lane III, purified MAb fraction.

Purification of recombinant mouse Fab fragment

Protein G has a low affinity site for the Fab region (binding to C_H1 domains of heavy chains bound to C_K light chains). Consequently, Protein G affinity purification can sometimes be used for the purification of Fab and $F(ab')_2$ fragments. Figure 5 shows the purification of recombinant mouse Fab fragments, expressed in *E. coli*, in a single affinity purification step using Protein G Sepharose 4 Fast Flow.

Medium: Protein G Sepharose 4 Fast Flow
Sample: Recombinant mouse Fab, expressed in *E. coli*. 15 ml, centrifuged.
Flow rate: 0.8 ml/min
Binding buffer: 50 mM Tris-HCl, 0.15 M NaCl, 0.05% Tween™, pH 7.4
Elution buffer: 0.2 M HAC, pH 2.8

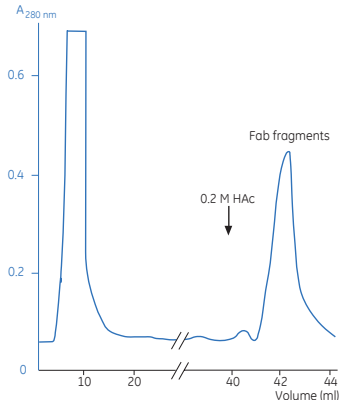


Fig 5. Purification of recombinant mouse Fab fragments, expressed in *E. coli*.

Ordering information

Product	Quantity	Code No.
Protein G Sepharose 4 Fast Flow	5 ml	17-0618-01
Protein G Sepharose 4 Fast Flow	25 ml	17-0618-02
Protein G Sepharose 4 Fast Flow	200 ml	17-0618-05
Protein G	5 mg	17-0619-01

Related products

HiTrap Protein G HP	5 × 1 ml	17-0404-01
HiTrap Protein G HP	2 × 1 ml	17-0404-03
HiTrap Protein G HP	1 × 5 ml	17-0405-01
HiTrap Protein G HP	5 × 5 ml	17-0405-03
MAbTrap™ Kit	1 kit	17-1128-01
HiTrap IgM Purification HP	5 × 1 ml	17-5110-01
HiTrap IgY Purification HP	1 × 5 ml	17-5111-01
Ab SpinTrap™	50 × 100 µl	28-4083-47
Protein G HP MultiTrap™	4 × 96-well plates	28-9031-35
Ab Buffer Kit	1	28-9030-59

Literature

Antibody Purification Handbook	1	18-1037-46
Affinity Chromatography Handbook	1	18-1022-29
Affinity Columns and Media, Selection Guide	1	18-1121-86
Convenient Protein Purification, HiTrap Column Guide	1	18-1129-8

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

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