Protein G **Sepharose** 4 Fast Flow

AFFINITY CHROMATOGRAPHY

DOMINIQUE DUTSCHER SAS

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, complementing the different Protein A Sepharose resins available from Cytiva
- No specific albumin binding
- Optimized homogeneous recombinant ligand
- High capacity



Fig 1. Protein G Sepharose 4 Fast Flow 5 mL and 25 mL.

Resin characteristics

Protein G is immobilized by the well-documented CNBr method on Sepharose 4 Fast Flow, a 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 binding capacity depends upon several factors, such as flow rate during sample application, and sample concentration.

Table 1 shows the total 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.

Stability

Protein G Sepharose 4 Fast Flow maintains the IgG binding capacity and recovery after storage in commonly used aqueous buffers and denaturants such as 6 M guanidine hydrochloride and 6 M urea.

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

Table 1. The total IgG capacity of Protein G Sepharose 4 Fast Flow for various species (evaluation performed at Cytiva[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

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.

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

Resin	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.

Operation and regeneration

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

To pack the resin, first wash away the ethanol solution with distilled water (pH 7) on a sintered glass filter or similar. While the resin is still on the filter, resuspend it in binding buffer (e.g., 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 resin, samples should be pretreated (e.g., centrigufation 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

Matrix	Cross-linked agarose, 4%, spherical
Particle size, d_{50v}[1]	~ 90 µm
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)	~ 17 000
pI of ligand	4.4
Ligand concentration	~ 2 mg protein G/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)[2,3]
Total binding capacity[4]	≥ 20 mg human IgG/mL resin
pH stability, operational[5]	3 to 9[6]
pH stability, CIP[7]	2 to 10[6]
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
Sanitization	Sanitize the column with 70% ethanol
Working temperature	2˚C to 40˚C
Storage	20% ethanol, 2°C to 8°C

[1] Median particle size of the cumulative volume distribution.
[2] The pressure/flow characteristics describe 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 10/10-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 below 3 is sometimes required to elute strongly bound IgG species. However, protein ligands may hydrolyze at pH below 2.
[7] pH range where resin can be subjected to cleaning- or sanitization-in-place without significant change in function.

In choosing a flow velocity 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 (determine the exact amount experiementally) of 1 M Tris™-HCl (pH 9.0) per mL fraction 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 2°C to 8°C (to prevent microbial contamination).

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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 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 Sepharose 4 Fast Flow than to different kinds of Protein A Sepharose resins, the former resin 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).

Resin:	Protein G Sepharose 4 Fast Flow
Sample:	100 µL bovine serum, centrifuged and filtered (0.22 µm)
Bed dimensions:	1 × 1.2 cm, VT: 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

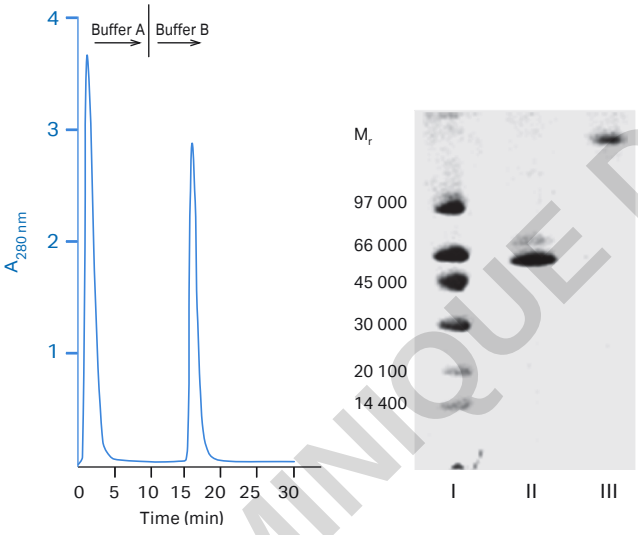


Fig 2. Protein G Sepharose 4 Fast Flow is an efficient resin for simple, rapid, and high-yield one-step separation of IgG from serum. SDS-PAGE of the non-reduced samples was run on PhastSystem™ using PhastGel™ 8–25 and Coomassie™ blue staining. Lane I, Low Molecular Weight Calibration Kit (Cytiva); Lane 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 resin 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
++ = resin binding
— = weak or no binding

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Resin:	Protein G Sepharose 4 Fast Flow
Sample:	100 µL mouse ascites in 100 µL binding buffer, centrifuged
Bed dimensions:	1 × 1.2 cm, VT: 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

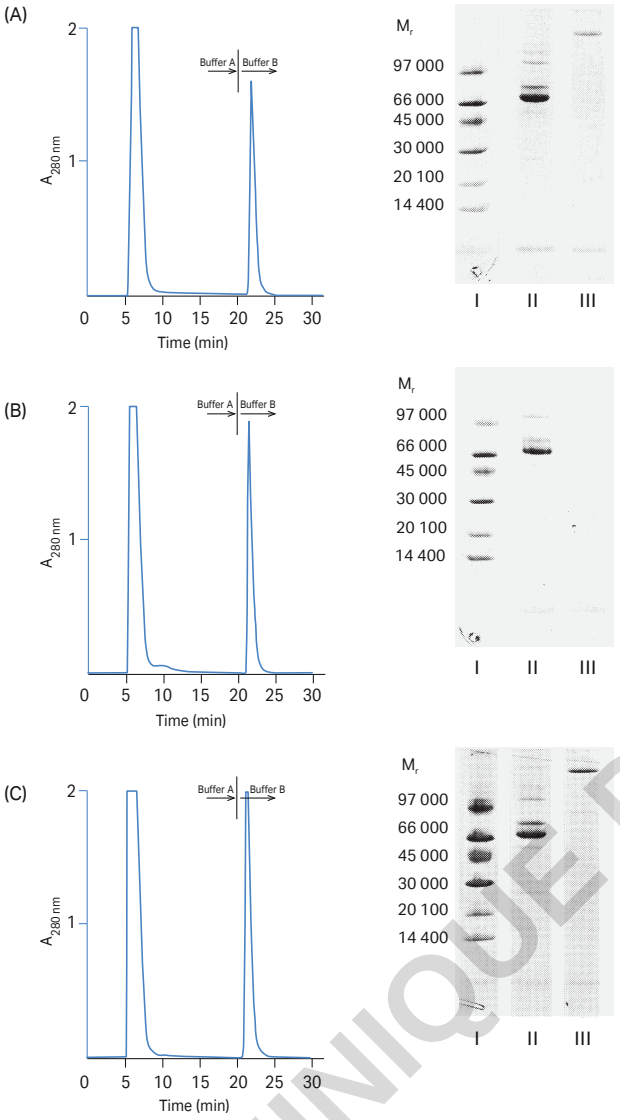


Fig 3. Protein G Sepharose 4 Fast Flow is used for the separation of monoclonal IgG from mouse ascites. The figure shows the separation of three different subclasses of mouse IgG: (A) IgG_1 (anti-CEA); (B) IgG_{2a} (anti-Apo A); (C) IgG_{2b} (anti-insulin). Adjacent insets show the SDS-PAGE of corresponding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

Resin:	Protein G Sepharose 4 Fast Flow
Sample:	10 µL cell culture fluid, filtered (0.22 µm)
Bed dimensions:	1 × 1.2 cm, VT: 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

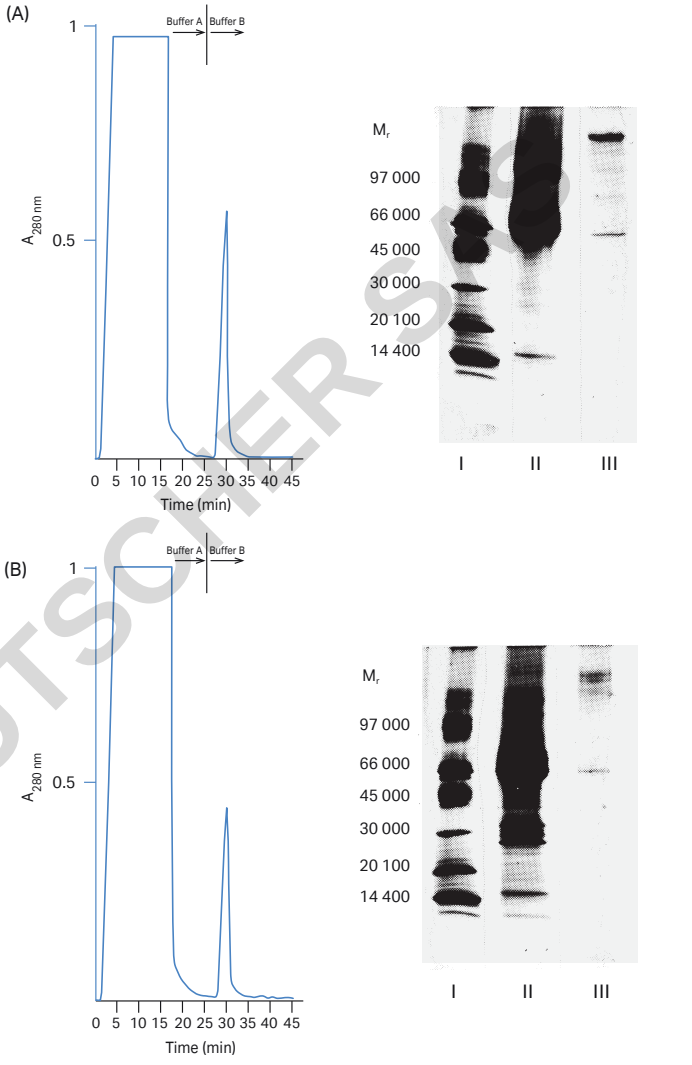


Fig 4. Purification of monoclonal IgG from rat hybridoma cell culture fluid. The figure shows the purification of two different subclasses of rat IgG: (A) IgG_{2a} (anti-CD4); (B) IgG_{2b} (anti-CD4). Adjacent insets show the SDS-PAGE of corresponding non-reduced samples. Samples were run on PhastSystem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

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.

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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 Cκ 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.

Resin:	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

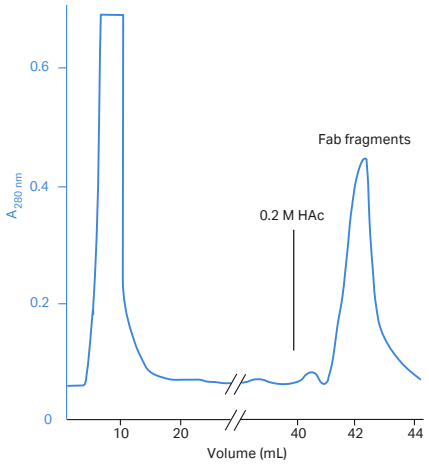


Fig 5. Purification of recombinant mouse Fab fragments, expressed in *E. coli.*

Ordering information

Product	Quantity	Product code
Protein G Sepharose 4 Fast Flow	5 mL	17061801
Protein G Sepharose 4 Fast Flow	25 mL	17061802
Protein G Sepharose 4 Fast Flow	200 mL	17061805
Protein G	5 mg	17061901

Related products

HiTrap Protein G HP	5 × 1 mL	17040401
HiTrap Protein G HP	2 × 1 mL	17040403
HiTrap Protein G HP	1 × 5 mL	17040501
HiTrap Protein G HP	5 × 5 mL	17040503
MAbTrap™ Kit	1 kit	17112801
HiTrap IgM Purification HP	5 × 1 mL	17511001
HiTrap IgY Purification HP	1 × 5 mL	17511101
Ab SpinTrap™	50 × 100 µL	28408347
Protein G HP MultiTrap™	4 × 96-well plates	28903135
Ab Buffer Kit	1	28903059

Literature

Antibody Purification Handbook	18103746
Affinity Chromatography Handbook	18102229
Affinity Columns and Media, Selection guide	18112186
Convenient Protein Purification, HiTrap Column guide	18112981

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