GE Healthcare
Life Sciences

Instructions 71-7083-00 AI **Affinity media**

Protein G Sepharose™ 4 Fast Flow

Protein G Sepharose 4 Fast Flow is protein G from GE Healthcare, immobilized by the CNBr method to Sepharose 4 Fast Flow.

Protein G binds to the Fc region of IgG from a variety of mammalian species. Protein G Sepharose 4 Fast Flow may be used to isolate and purify classes, subclasses and fragments of immunoglobulins from any biological fluid or cell culture medium. Since only the Fc region is involved in binding, the Fab region is still available for binding antigen. Hence, Protein G Sepharose 4 Fast Flow is extremely useful for isolation of immune complexes.

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 IgG_3 and mouse IgG_1, see Table 1.

Protein G Sepharose 4 Fast Flow belongs to the BioProcess™ Media family. BioProcess Media are separation media developed, made and supported for industrial scale – especially the manufacture of health care products. With their high physical and chemical stability, very high batch-to-batch reproducibility, and Regulatory Support File back-up, BioProcess Media are ideal for all stages of an operation – from process development through scale-up and into production.

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Table 1. Relative binding strengths of antibodies from various species to protein G and protein A 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 G binding	Protein A 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[1]	IgG_1	++++	+
	IgG_{2a}	++++	++++
	IgG_{2b}	+++	+++
	IgG_3	+++	++
	IgM	-	Variable
Pig		+++	+++
Rabbit		+++	++++
Rat[2]	IgG_1	+	-
	IgG_{2a}	++++	-
	IgG_{2b}	++	-
	IgG_3	++	+
Sheep	++		+/-

1 *IgG_1 from mouse binds more strongly to protein G than to protein A.*
2 *Note that IgG from rat binds to protein G copuled to Sepharose 4 Fast Flow.*

++++ = strong binding; ++ = medium binding; – = weak or no binding

Recombinant protein G, M_r 17 000, from GE Healthcare 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 4.1 and the pH stability 2 to 10.

Table 2. Characteristics of Protein G Sepharose 4 Fast Flow

Ligand density:	~2 mg protein G/ml drained medium
Dynamic binding capacity[1]:	~18 mg human IgG/ml drained medium
Matrix:	Spherical, 4% highly cross-linked agarose
Average particle size:	90 µm (45 to 165 µm)
Recommended flow rate[2]:	50 to 300 cm/h
Max operating backpressure:	0.1 MPa (1 bar, 14 psi)
pH stability[3]	
Long term:	3 to 9
Short term:	2 to 10
Chemical stability:	The IgG binding capacity and recovery was maintained after storage for: a) 7 days at 37°C in: 1 M acetic acid pH 2.0, 20 mM sodium phosphate, 1% SDS, pH 7. 0, 6 M guandine-HCl, 70% ethanol b) 2 hours at room temperature in: 0.1 M HCl, pH 1.0, 8 M urea, pH 10.5, 0.1 M Glycine-NaOH, pH 11
Physical stability:	Negligible volume variation due to changes in pH or ionic strength
Sanitization:	Sanitize the column with 70% ethanol
Storage:	20% etanol at 2°C to 8°C

1 The dynamic capacity was calculated as the amount of human IgG bound to the medium before the flow through exceeded 1% of the absorbance of the in-going solution. 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 might be considerable deviations in binding capacity for different immunoglobulins derived from the same species, even if they are of the same subclass.

2 Linear flow rate = $\frac{\text{volumetric flow rate (cm}^3\text{/h)}}{\text{column cross-sectional area (cm}^2\text{)}}$

3 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 of regeneration, cleaning in place and sanitization.
pH below 3 is sometimes required to elute strongly bound Ig's. However, protein ligands may hydrolyse at very low pH.

1 Preparing the chromatography medium

Protein G Sepharose 4 Fast Flow is supplied preswollen in 20% ethanol. Prepare a slurry by decanting the 20% ethanol solution and replace it with binding buffer in a ratio of 75% settled medium to 25% buffer. The binding buffer should not contain agents which significantly increase the viscosity. The column may be equilibrated with viscous buffers at reduced flow rate after packing is completed.

For batch procedures remove the ethanol by washing the medium on a medium porosity sintered glass funnel.

2 Packing Sepharose Fast Flow

1 Equilibrate all material at the temperature at which the chromatography will be performed.

2 De-gas the medium slurry.

3 Eliminate air from the column dead spaces by flushing the end pieces with binding buffer. Make sure no air has been trapped under the column net. Close the column outlet with a few centimetres of binding buffer remaining in the column.

4 Pour the slurry into the column in one continuous motion. Pouring the slurry down a glass rod held against the wall of the column will minimise the introduction of air bubbles.

5 Immediately fill the remainder of the column with binding buffer. Mount the column top piece onto the column and connect the column to a pump.

6 Open the bottom outlet of the column and set the pump to run at the desired flow rate. Ideally, Fast Flow media are packed at a constant pressure not exceeding 1 bar (0.1 MPa) in XK columns. If the packing equipment does not include a pressure gauge, use a packing flow rate of at least 400 cm/h (15 cm bed height, 25°C, low viscosity buffer).
If the recommended pressure or flow rate cannot be obtained, use the maximum flow rate the pump can deliver. This should also give a reasonably well-packed gel.

Note: *Do not exceed 75% of the packing flow rate in subsequent purification procedures*.

7 Maintain the packing flow rate for 3 bed volumes after a constant bed height is reached.

3 Using an adapter

Adapters should be fitted as follows:

1 After the medium has been packed as described above, close the column outlet and remove the top piece from the column. Carefully fill the rest of the column with distilled water to form an upward meniscus at the top.

2 Insert the adapter an angle into the column, ensuring that no air is trapped under the net.

3 Make all tubing connections at this stage. There must be a bubble-free liquid connection between the column and the pump and column.

4 Slide the plunger slowly down the column so that the air above the net and in the capillary tubings is displaced by buffer.

5 Lock the adapter in position, open the column outlet and start the flow of buffer. Pass buffer through the column at the packing flow rate until the medium bed is stable. Re-position the adapter on the medium surface as necessary.

The column is now packed and equilibrated and ready for use.

4 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 3.

However, the total capacity depends upon several factors, such as the flow rate during sample application, the sample concentration and binding buffer. Table 3 shows the total capacity under defined conditions for IgG from some species.

Table 3. The total IgG capacity of Protein G Sepharose 4 Fast Flow, under defined conditions[1], for various species. (Work from GE Healthcare)

Species	**Total IgG capacity (mg/ml drained medium)**
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 medium packed in a 1 × 10 cm column at a linear flow rate 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.*

5 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, we recommend the addition of 60 to 200 µl/ml eluate of 1 M Tris-HCl, pH 9.0, to neutralize the eluted fractions.

6 Regeneration

After elution, the medium 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.

7 Cleaning-in-place (CIP)

Remove strongly bound hydrophobic proteins, lipoproteins and lipids by washing the column with a non-ionic detergent (e.g. Triton™ X-100), 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.

8 Sanitization

Sanitization reduces microbial contamination of the chromatography medium 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.

9 Storage

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

10 More information

If you have further questions about Protein G Sepharose 4 Fast Flow, please visit:

www.gelifesciences.com/protein-purification

GE Healthcare technical support portal
www.gelifesciences.com/purification_techsupport
or contact your local GE Healthcare representatative.

11 Ordering information

Product	Pack size	Code No.
Protein G Sepharose 4 Fast Flow[1]	5 ml	17-0618-01
	25 ml	17-0618-02
	200 ml	17-0618-05

Related Products

HiTrap™ Protein G HP	1 ml × 5	17-0404-01
HiTrap Protein G HP	1 ml × 2	17-0404-03
HiTrap Protein G HP	5 ml × 1	17-0405-01
MAbTrap™ Kit	1 kit	17-1128-01
Ab SpinTrap™	100 µl x 50	28-4083-47
Protein G HP SpinTrap	100 µl x 16	28-9031-34
Protein G HP MultiTrap™	4 x 96-well plates	28-9031-35
Ab Buffer Kit	1 kit	28-9030-59
Protein G	5 mg	17-0619-01

Literature

Antibody Purification Handbook	1	18-1037-46
Solutions for antibody purification, Selection Guide	1	28-9330-94
Affinity Chromatography Handbook	1	18-1022-29
Affinity Columns and Media, Product profile	1	18-1121-46
Convenient Protein Purification, HiTrap Column Guide	1	18-1129-81

1 Larger quantities are available. Please contact GE Healthcare for more information.

For contact information for your local office, please visit:
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