

Protein A Sepharose 4 Fast Flow

Protein A Sepharose™ 4 Fast Flow is protein A immobilized by the CNBr method to Sepharose 4 Fast Flow.

Protein A binds to the Fc region of immunoglobulins through interaction with the heavy chain. The binding of protein A has been well documented for IgG from a variety of mammalian species and for some IgM and IgA as well.

Protein A Sepharose 4 Fast Flow has been used as a powerful tool to isolate and purify classes, subclasses and fragments of immunoglobulins from biological fluids and from cell culture media. Since only the Fc region is involved in binding, the Fab region is available for binding antigen. Hence, Protein A Sepharose 4 Fast Flow is extremely useful for isolating immune complexes.

Protein A 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.

Large quantities can be delivered at short notice.

Table 1. Medium characteristics.

Ligand density:	~6 mg protein A/ml drained medium
Dynamic binding capacity*:	35 mg human IgG/ml drained medium
Bead structure:	4% highly cross-linked agarose
Bead size range:	45–165 μm
Average bead size:	90 μm
Max. linear flow rate**:	>1300 cm/h at 25 °C, XK 16/20 column, 5 cm bed height
pH stability***	
Long term:	3–9
Short term:	2–10
Working:	2–9
Chemical stability:	The IgG binding capacity and recovery was maintained after storage for: (a) 7 days at 40 °C in: 3 M NaSCN, 6 M guanidine-HCl, 0.1 M glycine, pH 3.0, 70% ethanol. (b) 10 hours at 23 °C in: 8 M urea, 0.1 M glycine, pH 11 5.8 M acetic acid, pH 2.0
Physical stability:	Negligible volume variation due to changes in pH or in ionic strength.
Sanitization:	Sanitize the packed column with 2% Hibitane/20% ethanol or with 70% ethanol
Storage:	+4–8 °C in 20% ethanol

* The binding capacity was estimated in a 0.7×5 cm column at a linear flow rate of 300 cm/h under the following conditions:
Binding buffer: 0.1 M phosphate buffer, pH 7.0
Eluting buffer: 0.1 M citrate, pH 3.0.
2.8 times excess IgG of the binding capacity was applied.
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.

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

***Complete data on the stability of protein A 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 gel 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, see later.

pH below 3 is sometimes required to elute strongly bound Ig's. However, protein ligands may hydrolyse at very low pH.

Preparing the medium

Protein A 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.

Packing Sepharose Fast Flow

1. Equilibrate all material to 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 minimize 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 medium 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, the maximum flow rate, see Table 1, is typically employed during packing.

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 column.

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

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

Using an adaptor

Adaptors 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 binding buffer to form an upward meniscus at the top.
2. Insert the adaptor at an angle into the column, ensuring that no air is trapped under the net.
3. Make all tubing connectors at this stage. There must be a bubble-free liquid connection between the column and the pump.

4. Slide the plunger slowly down the column so that the air above the net and in the capillary tubings is displaced by binding buffer. Valves on the inlet side of the column should be turned in all directions during this procedure to ensure that air is removed.
5. Lock the adaptor in position on the medium surface, open the column outlet and start the buffer flow. Pass buffer through the column at the packing flow rate until the medium bed is stable. Re-position the adaptor on the medium surface as necessary.

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

Binding

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

As a general method, we recommend 20 mM sodium phosphate, pH 7.0 or 50 mM Tris buffer, pH 7.0 as binding buffer.

The binding capacity of Protein A Sepharose 4 Fast Flow depends on the source of the particular immunoglobulin. However, the dynamic capacity depends upon several factors, such as the flow rate during sample application, the sample concentration and binding buffer.

Note: There might be considerable deviations in binding capacity for different immunoglobulins derived from the same species, even if they are of the same subclass.

Elution

To elute IgG from Protein A Sepharose 4 Fast Flow, it is normally necessary to lower the pH to about 3.0, depending on the sample.

As a general method, we recommend 0.1 M glycine buffer, pH 3.0 or 0.1 M citric acid, pH 3.0 as elution buffer.

To elute very strongly binding IgG it may be necessary to lower the pH below 3.0.

As a safety measure to preserve the activity of acid labile IgGs, we recommend adding 60–200 µl, of 1 M Tris-HCl, pH 9.0, to neutralize the eluted fractions.

As an alternative, 3 M potassium isothiocyanate can be used for elution.

Regeneration

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

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

Cleaning-in-place (CIP)

Remove precipitated or denatured substances by washing the column with 2 column volumes of 6 M guanidine hydrochloride. Immediately re-equilibrate with at least 5 bed volumes of binding buffer.

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 binding buffer.

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

Sanitization

Sanitization reduces microbial contamination of the medium bed to a very low level.

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

Alternatively, equilibrate the column with 70% ethanol and let it stand for 12 hours.

Re-equilibrate the column with at least 5 bed volumes of sterile binding buffer.

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

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

Storage

All bulk media products are supplied in suspension in 20% ethanol. For longer periods of storage, keep the medium at 4–8 °C in a suitable bacteriostat, e.g. 20% ethanol. The medium must not be frozen.

Ordering information

Product	Pack size	Code No.
Protein A Sepharose 4 Fast Flow	5 ml	17-0974-01
Protein A Sepharose 4 Fast Flow	25 ml	17-0974-04

Further information

For additional information, including Regulatory Support File contact your local Amersham Biosciences representative.

The Antibody Purification Handbook, code no 18-1037-46 and the Affinity Chromatography Handbook, code no 18-1022-29 are useful for more information.

Check also *www.chromatography.amershambiosciences.com* for more information and latest news.

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