GE Healthcare

Instructions 71-5027-85 AC **Affinity media**

nProtein A Sepharose™ 4 Fast Flow

nProtein A Sepharose 4 Fast Flow is protein A immobilized by the CNBr method to Sepharose 4 Fast Flow. The medium is manufactured without using any animal-derived components.

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.

nProtein 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, nProtein A Sepharose 4 Fast Flow is extremely useful for isolating immune complexes.

nProtein 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 healthcare products. With their high physical and chemically stability, very high batch-to-batch reproducibility, and Regulatory Support File backup, BioProcess Media are ideal for all stages of an operation – from process development through scaleup 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[1]:	35 mg human IgG/ml drained medium
Bead structure:	4% highly cross-linked agarose
Bead size range:	45 to 165 µm
Average bead size:	90 µm
Recommended flow rate[2]:	50 to 300 cm/h
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 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:	2°C to 8°C in 20% ethanol

1 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.

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

[3] 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 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, see later. pH below 3 is sometimes required to elute strongly bound Igs. However, protein ligands may hydrolyze at very low pH.

Contents

1 Preparing the medium

This is a general procedure that may be used for most Sepharose media. Prepare a slurry with the binding buffer (see buffer recommendations under "Binding"), in a ratio of 75% settled medium to 25% buffer. Settled medium is defined as medium settled by gravity (including centrifuge-aided) – it does not refer to medium packed in a column. The binding buffer should not contain agents that significantly increase the viscosity such as 20% glycerol or 8 M urea. The column may be equilibrated with viscous buffers at reduced flow rates after packing is complete. The column may also be packed in distilled water and then equilibrated with binding buffer prior to the chromatography experiment. Always refer to the instructions supplied with the empty column to check for chemical compatibility and tolerance to maximum pressure and/or flow rate.

Note: *nProtein A Sepharose 4 Fast Flow is supplied preswollen in 20% ethanol.*

2 Packing Sepharose Fast Flow

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

2 De-gas the medium slurry. (This step is optional but may be helpful if air bubbles persist in the packed bed.)

3 Eliminate air from the column dead spaces by flushing the end pieces with buffer. Make sure no air has been trapped under the column net. Close the column outlet and allow a few centimeters of buffer to remain in the column.

4 Packing with a reservoir attached to the top of the column is highly recommended. Using a reservoir will eliminate the need to pour the media in multiple steps. It will also provide a high efficiency pack. Check the instructions supplied with the empty column for proper assembly of the reservoir.

5 Pour the slurry into the column (and reservoir, if used) in one continuous motion without stopping. Pouring the slurry down a glass rod held against the wall of the column will minimize the introduction of air bubbles.

6 Immediately fill the remainder of the column (and reservoir, if used) with buffer, mount the column-top piece (or the adapter) onto the column and connect the column to a pump.

7 Open the bottom outlet of the column and set the pump to run at the desired flow rate. Ideally, Fast Flow media are packed in XK columns at a constant pressure not exceeding 1 bar (0.1 MPa). If the packing equipment does not include a packing 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. DO NOT overpressure the column (see column specifications).

Note: *If you have packed at the maximum linear flow rate, do not exceed 75% of this in subsequent chromatographic procedures.*

8 Maintain the packing flow rate for 3 bed volumes after a constant bed height is reached. On the outside of the column mark the level of the packed bed.

3 Using an adapter

1. After the medium has been packed as described above, close the column outlet and remove the top piece (and reservoir, if used) from the column. Be sure to work quickly at this stage; with pressure removed, the medium may begin to expand upwards. This is normal and will be compensated for below. Carefully fill the rest of the column with buffer to form an upward meniscus at the top of the open column.
2. Insert the adapter 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 bubblefree liquid connection between the column and the pump.
4. Slide the adapter slowly down the column so that the air above the net and in the capillary tubings is displaced by binding buffer. Depress the adapter into the packed bed down to the mark on the outside of the column. This method of compressing the bed slightly is normal and will not disturb the packing.
5. Lock the adapter in position on the medium surface, open the column outlet and start the buffer flow. Pass the buffer through the column at the packing flow rate until the medium bed is stable. Reposition the adapter on the medium surface as necessary.

The column is now packed and ready for use. If packing in distilled water or in a low-viscosity buffer, equilibrate with three column volumes of binding buffer.

4 Binding

IgG from most species binds nProtein 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 nProtein 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.*

5 Elution

To elute IgG from nProtein 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 the 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, per ml fraction to neutralize the eluted fractions.

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

6 Regeneration

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

7 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 column volumes of binding buffer. Remove strongly bound hydrophobic proteins, lipoproteins and lipids by washing the column with a non-ionic detergent, e.g. 0.1% Triton™ X-100 at 37 °C for one minute. Immediately re-equilibrate with at least 5 column 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 column volumes of binding buffer.

8 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.

9 Storage

All bulk media products are supplied in suspension in 20% ethanol. For longer periods of storage, keep the medium at 2°C to 8°C in a suitable bacteriostat, e.g. 20% ethanol. The medium must not be frozen.

10 Further information

For additional information, including Regulatory Support Files contact your local GE Healthcare representative.

Useful information is also available in the Antibody Purification Handbook, see Ordering Information.

Visit our website *www.gelifesciences.com/protein-purification* for more information.

11 Ordering information

Product	Pack size	Code No.
nProtein A Sepharose 4 Fast Flow	5 ml	17-5280-01
	25 ml	17-5280-04
Related Literature		
Antibody Purification Handbook	1	18-1037-46
Affinity Chromatography columns and media product profile	1	18-1121-86

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