cytiva

Protein A **Sepharose** CL-4B

Affinity media

Instructions for Use

Introduction

Protein A Sepharose™ CL-4B is protein A immobilized by the CNBr method to Sepharose CL-4B.

Protein A binds to the Fc region of immunoglobulins through interactions 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 CL-4B 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 CL-4B is extremely useful for isolating of immune complexes.

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Table 1. Medium characteristics.

Ligand density:	≈ 3 mg protein A/mL drained medium
Available binding capacity[1]:	≈20 mg human IgG/mL drained medium
Bead structure:	4% cross-linked agarose
Bead size range:	45 to 165 µm
Mean bead size:	90 µm
Max linear flow rate[2]:	150 cm/h at 25°C, HR 16/10 columns, 5 cm bed height
pH stability[3].	
Long term:	3 to 9
Short term:	2 to 10
Chemical stability:	All commonly used aqueous buffers, 6 M guanidine-hydrochloride, 8 M urea.
Physical stability:	Negligible volume variation due to changes in pH or ionic strength.
Sanitization:	Sanitize the packed column with 2% Hibitane/20% ethanol or with 70% ethanol.

1 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 out 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.

Protein A may hydrolyse at low pH.

1 Preparing the medium

Protein A Sepharose CL-4B is supplied lyophilized in the presence of water soluble stabilization agents. These agents must be washed away in distilled water. Use the following procedure to wash the medium. Weigh out the required amount of lyophilized powder (1 g powder gives about 4 to 5 mL final volume of medium) and suspend it in distilled water. Suspend by gentle swirling – do not vortex as this may fracture the beads. The medium swells immediately and should be washed on a sintered glass filter. The sintered glass filter (also called a ground glass filter) should be of medium grade G3 or G4 type. Use approximately 200 mL distilled water per gram of powder, added in several aliquots. If this type of filter is not available, wash the medium by allowing it to settle by gravity and decanting off the supernatant. Introduce fresh distilled water in approximately the same quantity as the supernatant that was drawn off and resuspend the medium. Then allow the medium to settle as above. Repeat this procedure a total of three times using fresh distilled water each time. Rapid settling of the medium may be achieved by using a low speed centrifuge (use less than 500 × g to avoid crushing the medium). Please note that once the medium is swollen it should not be re-dried. Prepare a slurry with binding buffer, in a ratio of 75% settled medium to 25% buffer.

2 Packing Sepharose CL-4B medium

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

Step	Action
2	De-gas the medium slurry.
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 with a few centimeters of 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 buffer, mount the column top piece onto the column and connect the column to the pump.
6	Open the bottom outlet of the column and set the pump to run at the desired flow rate. This should be at least 133% of the flow rate to be used during subsequent chromatographic procedures. However, the maximum flow rate, see *Table 1, on page 4*, is typically employed during packing. ***Note:*** *If you have packed at the maximum linear flow rate, do not exceed 75% of this in subsequent chromatographic procedures.*
7	Maintain the packing flow rate for 3 bed volumes after a constant bed height is reached.

Using an adapter

Adapters should be fitted as follows:

Step	Action
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 buffer to form an upward meniscus at the top.
2	Insert the adapter at 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.
4	Slide the plunger slowly down the column so that the air above the net and in the capillary tubings is displaced by eluent. 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 adapter in position on the medium surface. Open the column outlet and start the eluent flow. Pass eluent through the column at the packing flow rate until the bed is stable. Re-position the adapter on the medium surface as necessary.

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

3 Binding

IgG from most species binds Protein A Sepharose CL-4B 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 buffers.

The binding capacity of Protein A Sepharose CL-4B 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.*

4 Elution

To eluate IgG from Protein A Sepharose CL-4B, 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 IgGs 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 to 200 µL of 1M 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.

5 Regeneration

After elution, the medium should 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 this regeneration procedure. These can be removed by Cleaning-In- Place procedures.

6 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 binding buffer.

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

7 Sanitization

Sanitization reduces microbial contamination of the medium to a very low level. Equilibrate the medium with a buffer containing 2% hibitane digluconate and 20% ethanol. Allow to stand for 6 hours.

Alternatively, equilibrate the medium 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 of sanitization procedures described above.

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

8 Storage

Lyophilized Protein A Sepharose CL-4B should be kept below 8°C. Swollen medium should be stored in neutral pH at 2°C to 8°C in the presence of a bacteriostat, e.g. 20% ethanol. Swollen medium must not be frozen.

9 Ordering information

Product	Pack size	Code No.
Protein A Sepharose CL-4B	1.5 g	17078001
Literature		
Antibody Purification Handbook	1	18103746

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For local office contact information, visit cytiva.com/contact

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