



Protein A **Sepharose** CL-4B

Affinity media

Instructions for Use

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1 Abstract

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.

Table 1. Medium characteristics.

Ligand density:	~3 mg protein A/mL drained medium
Available capacity ¹ :	~20 mg human IgG/mL drained medium
Bead structure:	4% cross-linked agarose
Bead size range:	45–165 μm
Mean bead size:	90 μm
Max linear flow rate ² :	150 cm/h at 25°C, HR 16/10 column, 5 cm bed height
pH stability ³ :	
Long term:	3–9
Short term:	2–10
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 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 to the best of 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.

Protein A may hydrolyse at low pH.

2 Preparing the medium

Protein A Sepharose CL-4B 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 procedure remove the ethanol by washing the medium on a medium porosity sintered glass funnel.

3 Packing Sepharose CL-4B

Step	Action
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| 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 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. |

Step	Action
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| 5 | Immediately fill the remainder of the column with 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. 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.

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| 7 | Maintain the packing flow rate for 3 bed volumes after a constant bed height is reached. |
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4 Using an adaptor

Adaptors should be fitted as follows:

Step	Action
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| 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. |
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Step Action

- 2** Insert the adaptor 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 adaptor 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 adaptor on the medium surface as necessary.
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The column is now packed and equilibrated and ready for use.

5 Binding

IgG from most species binds Protein A Sepharose CL-4B at neutral pH and physiological 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.*

6 Elution

To elute 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 60–200 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.

7 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.

8 Cleaning in place (CIP)

Remove precipitated or denatured substances by washing the medium 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 medium 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 medium with 70% ethanol and let it stand for 12 hours. Re-equilibrate with at least 5 column volumes of binding buffer.

9 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 medium 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.

10 Storage

For longer periods of storage, keep Protein A Sepharose CL-4B at 4°C to 8°C in a suitable bacteriostat, e.g. 20% ethanol. The medium must not be frozen.

11 Further information

Check cytiva.com/protein-purification for more information. Useful information is also available in the Handbook 18103746, see *Ordering information*.

12 Ordering information

Product	Pack size	Code No.
Protein A Sepharose CL-4B	25 mL	17096303
Literature		
Antibody Purification Handbook	1	18103746

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