IFTA AG
Certified QMS according to
DIN EN ISO 9001:2000-12
Reg. No. IC 03214 034

Protein A Agarose
bulk material

Cat. No.	Amount
AC-309-5	5 ml
AC-309-10	10 ml
AC-309-25	25 ml
AC-309-50	50 ml

For *in vitro* use only
Quality guaranteed for 12 months
Store at 4°C. Do not freeze.

Description

Protein A resin allows batch or column purification of classes, subclasses and fragments of immunoglobulins from cell culture media and biological fluids. Recombinant Protein A is covalently bound to the matrix, avoiding protein loss and allowing re-use of the resin.

Form and Specifications

This product is supplied as a suspension in 20 % ethanol.

Degree of substitution: ~ 3 mg Protein A /ml resin
Binding capacity: ~ 25 mg human IgG / ml resin

General Procedure

Protein A consists of a single polypeptide chain which contains five highly homologous antibody-binding domains. The binding site is located on the Fc region of immunoglobulin. Protein A has affinity for IgG from a variety of mammalian species and for some IgA and IgM. Recombinant Protein A shares identical binding properties to IgG as the Cowan I strain of natural Protein A.

1. Removal of the storage solution

Wash the beads with 5 - 10 column volumes of distilled water to remove the storage solution.

Please note: For batch purification, remove the storage solution by washing the product on a medium porosity sintered glass funnel.

2. Equilibration of the Protein A Agarose resin

Equilibrate the column with 5 - 10 column volumes of binding buffer.

Binding buffer: IgG from most species binds at neutral pH. The buffers used most frequently are sodium phosphate (25 mM) or Tris (50 mM) at pH 7.0. Binding occurs through an induced hydrophobic frit and is promoted by addition of salts. At alkaline pH, the interaction between Protein A and the antibody is stronger. Other buffers used frequently are PBS (100 mM), NaCl (150 mM) at pH 7.2.

3. Application of the sample

Once the resin is equilibrated, the sample containing the immunoglobulin for purification is applied.

Please note:

- In some cases, a slight increase of contact time may facilitate binding.
- In some cases diluting the sample with binding buffer (1 : 1) before application is recommended to maintain the proper ionic strength and pH for optimal binding.
- Binding capacity can be affected by several factors such as sample concentration, binding buffer and flow rate during sample application.

4. Washing of the Protein A Agarose resin

Wash with the binding buffer until OD_{280} reaches baseline level.

5. Elution of the pure immunoglobulin

Elution is usually achieved at reduced pH. Depending on the sample it may be necessary to decrease pH below 3.0. Most immunoglobulins are eluted in glycine (100 mM) or citric acid buffer (100 mM) at pH 3.0.

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Please note: It is recommended to add 0.15 ml of a buffer at pH 9.0 (e.g 1 M Tris) per ml of purified immunoglobulin to neutralize the eluted fractions. A more drastic method uses potassium isothiocyanate (3 M) as elution buffer.

Column Packing

The following procedure describes how a column is packed from bulk affinity resins:

1. Shake the bottle manually to obtain a homogenous suspension of the affinity resin. Place a funnel in the column head and slowly pour the suspension down the walls of the column.

Please note: Add the suspension slowly to avoid the formation of bubbles. The suspension may also be degassed before being applied to the column.

Decant the product and discard most of the leftover liquid, leaving 1 cm above the column head to prevent drying out. This can be done either by passing the liquid through the column, or pipetting it from the top of the column.

2. Repeat the previous step until the desired column height is obtained.

3. Insert the adapter gently into the column head until it begins to displace the liquid.

Please note: Make sure no air is trapped under the net.

4. Add distilled water via the tubing until a constant height (corresponding to the height of the column) is achieved.

Please note: If the desired height is not achieved, repeat steps 1 through 4.

5. Once a constant height has been achieved, rinse the column with 5 volumes of distilled water for complete elimination of the storage solution.

6. Equilibrate the column with 5 - 10 column volumes of binding buffer.

Please note: It is recommended to de-gas all solutions prior to adding them to the column to avoid the formation of bubbles.

Recommended Conditions

Linear flow rate:	26 cm/h
Recommendes flow rate:	0.5 - 1.0 ml/min
Max. pressure:	2.6 psi (0.18 bar)