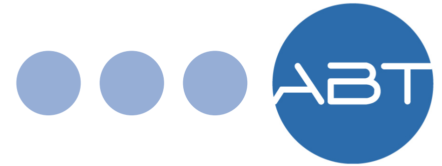
PROTEIN A AGAROSE BEADS



PROCEDURE FOR USE
Bulk Resins

DESCRIPTION

Resins are products that allow batch or column purifications of classes, subclasses and fragments of immunoglobulins from cell culture media and biological fluids. ^{r}Protein A is immobilized by means of covalent binding that avoids protein loss and allows for column re-use.

This product is supplied as a suspension of PROTEIN A Agarose Resin in 20% ethanol.

PROTEIN A Agarose Resin specifications:

Ligand density: ~ 3 mg ^{r}Protein A /ml resin.
Binding Capacity: ~ 25 mg human IgG / ml resin.

INSTRUCTIONS

^{r}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. ^{r}Protein A has affinity for IgG from a variety of mammalian species and for some IgA and IgM. The recombinant Protein A shares identical binding properties to IgG as the Cowan I strain of natural Protein A.

1. Elimination of the Preservative

Wash the beads with 5 - 10 column volumes of distilled water to eliminate the preservative.

Note: For batch purification remove the preservative by washing the product on a medium porosity sintered glass funnel.

2. Equilibration of the ^{r}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), pH 7.0. Binding occurs through an induced hydrophobic frit and is promoted by addition of salts. At alkaline pH, the interaction between the ^{r}Protein A and the antibody is stronger. Generally other buffers used are PBS (100 mM), NaCl (150 mM) pH 7.2.

3. Application of the Sample

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

Note: In some cases a slight increase of contact time may facilitate binding.

Note: Sometimes diluting simple 1:1 with binding buffer before application is advisable to maintain the proper ionic strength and pH for optimal binding.

Note: Binding capacity can be affected by several factors such as sample concentration, binding buffer and the flow rate during sample application.

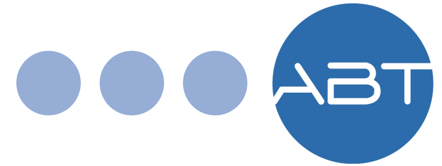
4. Washing of the ^{r}Protein A Agarose Resin

Wash with the binding buffer until the O.D. 280 nm reaches the baseline level.

5. Elution of the Pure Immunoglobulin

Elution is normally achieved at reduced pH and 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) pH 3.0.

C/ La Forja, 9 · 28850 · Torrejón de Ardoz · Madrid · SPAIN · Phone. +34 91 761 02 30/32 · Fax +34 91 675 74 44
5008 West Linebaugh Ave. Suite 44 · Tampa, FL 33624, USA · Phone 813 908 2589 · Fax 813 908 3190
info@abtbeads.com
www.abtbeads.com



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

6. Storage

Keep at +2ºC - +8ºC. Do not freeze.

COLUMN PACKAGING

1. Gently shake the resin bottle several times to obtain a homogeneous suspension of Protein A Agarose resin/preservative. Place a funnel in the head of column and slowly run the suspension down the walls of the column.

   Note: It is advisable to make the addition slowly to avoid the formation of bubbles. The product may also be degassed before added to the column.
   Decant the product and discard most of the leftover liquid, leaving 1 cm above the column head to prevent drying. This is done either by passing it through the column or pipetting it from the top of the column.

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

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

   Note: Make sure no air is trapped under the net.

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

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

5. When a constant height has been obtained, maintain the flow with the addition of 5 volumes of distilled water to completely eliminate the preservative.

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

   Note: It is advisable to previously de-gas all the solutions before adding to the column to avoid the formation of bubbles.

WORK RECOMMENDED CONDITIONS

LINEAR FLOW RATE	26 cm/h
RECOMMENDED FLOW RATE	0.5 - 1.0 ml/min
MAX. PRESSURE	2.6 psi (0.18 bar)

For laboratory use only. Not for use in diagnostic or therapeutic procedures.

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C/ La Forja, 9 · 28850 · Torrejón de Ardoz · Madrid · SPAIN · Phone. +34 91 761 02 30/32 · Fax +34 91 675 74 44
5008 West Linebaugh Ave. Suite 44 · Tampa, FL 33624, USA · Phone 813 908 2589 · Fax 813 908 3190
info@abtbeads.com
www.abtbeads.com