



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. ¹²⁵I-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 ¹²⁵I-Protein A /ml resin.

Binding Capacity: ~ 25 mg human IgG / ml resin.

INSTRUCTIONS

¹²⁵I-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. ¹²⁵I-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 ¹²⁵I-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 fit and is promoted by addition of salts. At alkaline pH, the interaction between the ¹²⁵I-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 sample 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 ¹²⁵I-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.



PROCEDURE FOR USE Bulk Resins

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 +2°C - +8°C. 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.

ABT GD PA RES Rev. 2008/B