Protocol

Antibody Binding Protocol

Utilizing Protein A Agarose Beads

Introduction

Protein A agarose Beads/Resins are important products used in Protein A affinity chromatography, an elemental technique in the purification of monoclonal antibodies. These isolated monoclonal antibodies are routinely used as effective therapeutic agents in treatment of many cancers. In these resins, Protein A is immobilized by covalent binding that allows batch or column purifications of classes, subclasses and fragments of immunoglobulins (antibodies) from cell culture media and biological fluids. Protein A consists of a single polypeptide chain containing five highly homologous antibody-binding domains. In these resins, purification with a high purity yield is possible because Protein A binds strongly to the Fc region of immunoglobulin. Specifically, Protein A has affinity for IgG from a variety of mammalian species and for some IgA and IgM. In addition, recombinant protein A shares identical binding properties to IgG as the Cowan I strain of natural Protein A. Here, we describe a procedure for packing of a column and purification of antibodies using Protein A agarose beads.

Materials

- Protein A Agarose Beads (GoldBio Catalog # P-400)
- Distilled water
- Purification columns
- Binding Buffer: Sodium phosphate, Tris, PBS or NaCl
- Elution Buffer: Glycine or Citric Acid

Storage and Handling

- Protein A Agarose Beads should be stored at 4°C. Do not freeze.

Method

Protein A Agarose Beads are supplied as a suspension of Protein A 4% Agarose Resin in 20% ethanol. This procedure avoids protein loss and allows for column re-use and may vary depending on the type of column used.

Column Packaging

1. Manually shake the bottle to obtain a homogeneous suspension of Protein A Agarose Resin preservative. Refer to Table 1 for recommended working conditions.

Table 1. Recommended working conditions.

Linear Flow Rate	26 cm/hour
Recommended Flow Rate	0.5-1.0 ml/minute
Maximum Pressure	2.6 psi (0.18 bar)

2. Place a sterile funnel in the head of the column and slowly run the suspension down the walls of the column.

   Note: Add the suspension slowly to avoid the formation of bubbles. The product may also be degassed before being added to the column.

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

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

5. 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.

6. 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 6.

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

8. Equilibrate the column with 5-10 column volumes of binding buffer. Proceed to step 2 in the Purification section.

   Note: Degas all the solutions before adding to the column to avoid bubble formation.

Purification

1. Eliminate the preservative by washing the beads with 5-10 column volumes of distilled water.

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

2. Add 5-10 column volumes of binding buffer at the temperature the purification will be performed to equilibrate the column. Refer to Table 2 for Protein A 4% Agarose Resin specifications.

Note: IgG from most species bind at a neutral pH. The buffers used most frequently are sodium phosphate (25mM) or Tris (50mM) 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. Additional buffers that may be used include PBS (100mM) and NaCl (150mM) pH 7.2.

Table 2. Protein A 4% Agarose Resin specifications

Ligand density	~ 3 mg Protein A/ml resin
Binding capacity	~ 25 mg human IgG/ml resin
Resin	4% highly cross-linked agarose beads

3. Once the resin is equilibrated, add the sample containing the immunoglobulin for purification. In some cases, a slight increase of contact time may facilitate binding.

Note: Occasionally, diluting the sample 1:1 with binding buffer before addition may provide the proper ionic strength and pH for optimal binding.

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

4. Wash the resin with binding buffer until the optical density (O.D.) at 280 nm reaches the baseline level.

5. Elution of the pure immunoglobulin is normally achieved by reducing the pH and, depending on the sample, it may be necessary to decrease the pH below 3.0.

Note: Most immunoglobulins are eluted in glycine (100mM) or citric acid buffer (100mM) at pH 2.5-3.0.

Note: It is recommended to add 0.15 ml of buffer at pH 9.0 (e.g. 1M Tris) per ml of purified immunoglobulin to neutralize the eluted fractions.

Note: A more drastic elution method requires the use of potassium isothiocyanate 3M as elution buffer.

6. Store at 2-8°C in a suitable bacteriostatic agent, e.g. 20% ethanol. **Do not freeze.**

Associated Products

- Protein A Agarose Beads (GoldBio Catalog # P-400)
- Phosphate Buffered Saline, PBS (GoldBio Catalog # P-271)
- Glycine (GoldBio Catalog # G-630)
- Tris (GoldBio Catalog # T-400)
- Plastic columns (GoldBio Catalog # P-301 or P-302)

References

Hober, S., Nord, K. and Linhult, M. (2007). Protein A chromatography for antibody purification. *Journal of Chromatography B,* 848(1), 40-47. Doi:10.1016/j.jchromb.2006.09.030

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