Protocol

Antibody Binding Protocol

Utilizing Protein G Agarose Beads

Introduction

Protein G agarose beads/resins, like Protein A agarose beads/resins, are routinely used in affinity chromatography, an elemental technique in the purification of monoclonal antibodies. Protein G is immobilized by covalent binding to the resin, allowing batch or column purifications of classes, subclasses, and fragments of immunoglobulins (antibodies) from cell culture media and biological fluids. In addition, Protein G, a bacterial cell wall protein first isolated from a *G. streptococcal* strain (G148), binds fragment crystallizable (Fc) regions and antigen-binding (Fab) fragments in immunoglobulins. Protein G contains 2 immunoglobulin binding sites, an albumin binding site, and a cell surface binding site. Conversely, recombinant Protein G, often made in *E. coli*, does not contain the albumin-binding domain or the cell wall and cell membrane binding domains ensuring maximum specific IgG binding capacity. Specifically, Protein G does not bind to human IgA, IgD or IgM. Furthermore, recombinant Protein G has a predicted molecular mass of approximately 21.6 kDa, but migrates with an apparent molecular mass of 32 kDa in SDS-PAGE. Importantly, the binding capacity of Protein G Agarose Resin depends on the source of the immunoglobulin. There might be deviations in binding capacities for different immunoglobulins derived from the same species, even if they are of the same subclass. Here, we describe a procedure for packing a column and purification of antibodies using Protein G agarose beads.

Materials

- Protein G Agarose Beads (GoldBio Catalog # P-430)
- Distilled water
- Purification Column
- Binding Buffer: Sodium phosphate, 25mM, pH 7.0.
- 1M Tris, pH 9.0
- Elution Buffer: Glycine or Citric Acid

Storage and Handling

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

Method

Protein G Agarose Beads are supplied as a suspension of Protein G HTC 4% Agarose Resin in 20% ethanol. The isoelectric point of Protein G is 4.1 and the pH stability ranges from 2 to 10.

Optimal binding occurs at a pH of 5.0, although binding is also favorable between 7.0 and 7.2. This procedure may vary depending on the type of column used.

Column Packing

1. Manually shake the Resin bottle to obtain a homogeneous suspension of Protein G Agarose Resin preservative.

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 the 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 the sample 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 1 for Protein G HTC 4% Agarose Resin specifications.

   Note: IgG from most species bind at a neutral pH. The buffer used most frequently is sodium phosphate (25mM) at a pH of 7.0.

Table 1. Protein G HTC 4% Agarose Resin specifications.

Ligand density	~ 3 mg Protein G/ml resin
Binding capacity	~ 20 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: 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. Wash 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 at a reduced 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.

6. Store at 2-8°C in a suitable bacteriostatic agent, e.g. 20% ethanol, for short term storage (1 week). **Do not freeze.**

Associated Products

- Protein G Agarose Beads (GoldBio Catalog # P-430)
- Glycine (GoldBio Catalog # G-630)
- Plastic columns (GoldBio Catalog # P-301 or P-302)
- Tris (GoldBio Catalog # T-400)

References

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