rProtein A Agarose Beads
PROCEDURE FOR USE

DESCRIPTION

rProtein A Agarose resins are products that allow batch or column purifications of classes, subclasses and fragments of immunoglobulins from cell culture media and biological fluids. 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 rProtein A Agarose Resin in 20% ethanol.
rProtein A Agarose Resin specifications:
Binding Capacity: ~25 mg human IgG / ml resin.

INSTRUCTIONS

rProtein 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. rProtein 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 rProtein 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 rProtein 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 rProtein 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.
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 rProtein 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)

TROUBLESHOOTING GUIDE

Problems and Solutions

Possible causes of problems that could appear during the purification protocol of immunoglobulins are listed below. The causes described are theoretical and it is always advisable to contact our team with your specific problem.
The table delineates the potential problems at each step in the protocol that might explain poor performance.

OBSERVATION	POSSIBLE CAUSES	RECOMMENDATION
TARGET PROTEIN NOT BOUND TO THE COLUMN	• Conditions in binding or elution are not the optimum ones.	• Optimize pH, flow, temperature as well as salt or ion concentration.
	• Channels have formed in column bed so loaded sample runs through column without interacting with Protein A.	• Re-pack column.
	• Column has been stored in recommended conditions alter previous usage.	• Always follow manufacturer recommendations.
	• The antibody to be purified has low affinity with Protein A.	• Look up bibliography on the subject and if that observation is true try an alternative way of purification.
	• Protease presence.	• Add protease inhibitors to sample loading/wash buffer. • Work at lower temperatures (such as 4° C) to minimize degradation.
THE ANTIBODY IS DEGRADED	• Antibody can be unstable in elution conditions.	• Follow usage instructions neutralizing the fractions of the eluted antibody.
ANTIBODY IS NOT DETECTED IN THE ELUTION PROCESS	• The IgG subclass doesn't bind to the resin.	• Use another affinity column to purify the antibody.
BUBBLES IN THE PRE-PACKED COLUMN	• Column poured and stored at one temperature, but used at another.	• Equilibrate the column in the same temperature conditions as in usage step.
	• There are air bubbles in sample or buffers.	• De-gas sample and buffers used.
COLUMN FLOW IS VERY SLOW	• There are air bubbles in sample or buffers that are blocking flow through pores.	• De-gas sample and buffers used.

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