BTS

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BTS Provides the Best tools for Protein Purification!

PROTEIN A AGAROSE BEADS

PROCEDURE FOR USE
Pre-packed columns

DESCRIPTION

Pre-Packed Columns are in ready-to-use format to isolate and purify classes, subclasses and fragments of immunoglobulins from cell culture media and biological fluids. Rapid purifications and high yield of purified immunoglobulin are obtainable by this method. [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:

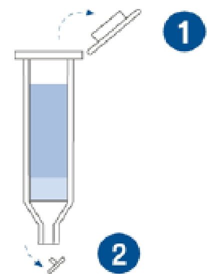
Ligand density: ~ 3 mg [r]Protein A /ml resin
Binding Capacity: ~ 25 mg human IgG / ml resin.

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.

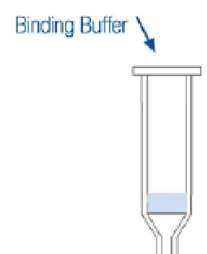
1. Elimination of the Preservative

Remove first the upper and then the lower cap of the column to allow elimination of the preservative by gravity flow.



2. Equilibration of the Pre-Packed Column

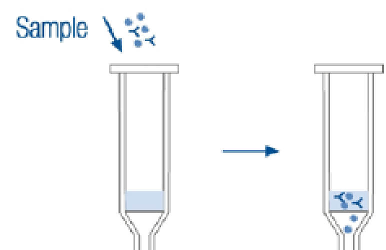
Equilibrate the column with 5 - 10 column bed volume of binding buffer. Mix manually inverting the Pre-Packed column. Add the binding buffer to the upper part of the column and make sure no air has been trapped.



Binding buffer: IgG from most species binds at neutral pH. The buffers most frequently used 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 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

Add the sample containing the immunoglobulin to be purified through the top of the column, keeping sample and resin in contact at least 15 minutes before removing the bottom cap.



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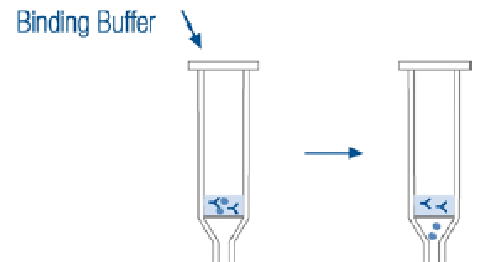
Note: In some cases a slight increase in contact time may facilitate binding.

Note: Pouring the sample down a glass rod held against the wall of the column will minimize the introduction of air bubbles. Sometimes diluting simple 1:1 with binding buffer before application to the column is advisable to maintain the proper ionic strength and pH for optimal binding.

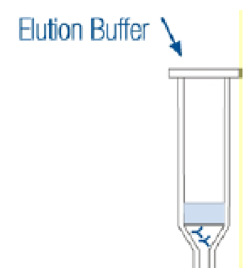
Note: Binding capacity can be affected by several factors such as sample concentration, binding buffer and the flow rate during sample application.

4. Washing of the Pre-Packed Column



Add the binding buffer through the top to eliminate all the proteins that have not been retained in the column. Mix manually inverting the Pre-Packed column. Wash the column with binding buffer until the O.D. 280 nm of the eluent 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: Mix manually inverting the Pre-Packed column. It is advisable to keep elution buffer and resin in contact at least 10 minutes before removing the bottom cap.

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.