

## Protein A Agarose Pre-packed Columns

Cat. No.	Amount
AC-309C-1	1 column (1 ml)
AC-309C-5	1 XL column (5 ml)

For *in vitro* use only  
 Quality guaranteed for 12 months  
 Store at 4°C. Do not freeze.

### Description

Pre-packed Protein A columns are ready-to-use for the isolation and purification of classes, subclasses and fragments of immunoglobulins from cell culture media and biological fluids. By this method, immunoglobulins can be purified rapidly and obtained in good yield. Recombinant Protein A is covalently bound to the matrix, avoiding protein loss and allowing re-use of the resin.

### Form and Specifications

This product is supplied as a suspension in 20 % ethanol.

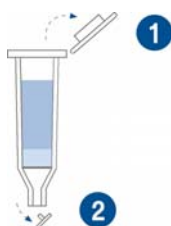
Degree of substitution: ~ 3 mg Protein A /ml resin  
 Binding capacity: ~ 25 mg human IgG / ml resin

### General Procedure

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. Protein A has affinity for IgG from a variety of mammalian species and for some IgA and IgM. Recombinant Protein A shares identical binding properties to IgG as the Cowan I strain of natural Protein A.

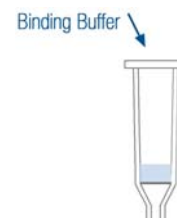
#### 1. Removal of the storage solution

First, remove the upper and then the lower cap of the column, to allow removal of the storage solution by gravity flow.



#### 2. Equilibration of the pre-packed column

Equilibrate the column with 5 - 10 column bed volumes of binding buffer. Add the binding buffer at the upper part of the column and make sure no air has been trapped. Mix manually inverting the pre-packed column.

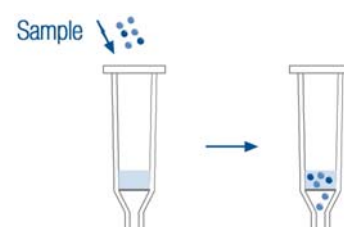


Binding buffer: IgG from most species binds at neutral pH. The buffers used most frequently are sodium phosphate (25 mM) or Tris (50 mM) at pH 7.0. Binding occurs through an induced hydrophobic fit and is promoted by addition of salts. At alkaline pH, the interaction between Protein A and the antibody is stronger. Other buffers used frequently are PBS (100 mM), NaCl (150 mM) at pH 7.2.

#### 3. Application of the sample

Bring the bottom cap of the column back in place.

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.



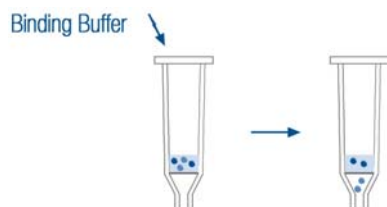
Please note:

- In some cases, a slight increase of contact time may facilitate binding.
- Pouring the sample down a glass rod held against the wall of the column will minimize the introduction of air bubbles. In some cases diluting the sample with binding buffer (1 : 1) before application is recommended to maintain the proper ionic strength and pH for optimal binding.
- Binding capacity can be affected by several factors such as sample concentration, binding buffer and the flow rate during sample application.

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### 4. Washing of the pre-packed column

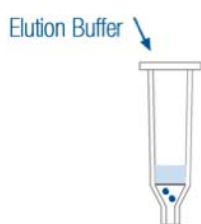
Add the binding buffer through the top to eliminate all the proteins that have not been retained in the



column. Mix manually inverting the pre-packed column. Wash the column with binding buffer until  $OD_{280}$  of the eluent reaches baseline level.

### 5. Elution of the pure immunoglobulin

Elution is usually achieved at reduced pH. 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) at pH 3.0. Mix manually inverting the pre-packed column. It is recommended to keep elution buffer and resin in contact for at least 10 minutes before removing the bottom cap.

*Please note:* It is recommended to add 0.15 ml of a buffer at pH 9.0 (e.g. 1 M Tris) per ml of purified immunoglobulin to neutralize the eluted fractions. A more drastic method uses potassium isothiocyanate (3 M) as elution buffer.