



## PROCEDURE FOR USE PROTEIN A RAPID RUN™ Cartridges

### DESCRIPTION

Protein A Rapid Run™ Cartridges are used to purify 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.

Cartridges are “ready to use” FPLC™ columns prepacked with Protein A Rapid Run™ for rapid purification of monoclonal and polyclonal antibodies. This cartridge can be used with an automated chromatography system, a peristaltic pump or with a syringe for manual processing.

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

### INSTRUCTIONS

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. The recombinant Protein A shares identical binding properties to IgG as the Cowan I strain of natural Protein A.

Cartridges (1 ml) can be operated with liquid chromatography systems (such as ÄKTA™ design systems) via standard 10-32 fittings with additional connectors (1/16" male). The recommended flow rate is 1 ml /min.

#### 1. Connect Cartridge to the chromatography system

Purge the pump with binding buffer. Assure that all air is displaced. Remove the snap-off end at the cartridge outlet and save it for further use. Remove the upper plug from the cartridge.

Fill the inlet port of the cartridge with several drops of buffer to remove air to form a positive meniscus. Start the pump and insert the fitting “drop-to-drop” into the cartridge port to avoid introducing air bubbles.

Wash the beads with at least 5 ml of distilled water to eliminate the preservative.

#### 2. Equilibration of the Cartridge

Equilibrate the cartridge with at least 5 ml 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 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

Once the cartridge is equilibrated, the sample containing the immunoglobulin is applied. Load the centrifuged or filtered sample onto the cartridge.



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**Note:** Filter the sample through a 0.45  $\mu\text{m}$  filter and / or centrifuge it immediately before the application to the cartridge. Sometimes diluting sample 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. In some cases a slight increase of contact time may facilitate binding. The medium has a binding capacity for human IgG of approximately 25 mg of IgG /ml medium.

### 4. Washing of the Cartridge

Wash the cartridge with binding buffer until the O.D. 280 nm reading is stable.

### 5. Elution of the Pure protein

Elution is normally achieved using 2 -5 ml of buffer at reduced pH most immunoglobulins are eluted in glycine (100 mM) or citric acid buffer (100 mM) pH 3.0. Depending on the sample it may be necessary to decrease pH below 3.0 and use a higher volume of buffer.

**Note:** It is recommended the addition of 50 -200  $\mu\text{l}$  of buffer pH 9.0 (e.g Tris 1 M) per ml of purified immunoglobulin to neutralize the eluted fractions. A more drastic method uses potassium isothiocyanate (3 M) as elution buffer.

**Note:** Conditions (volumes, times, temperatures) used for elution may vary. Eluates should be monitored (Bradford protein assay, SDS-PAGE or measure the absorbance at 280 nm to determine the yield of the eluted immunoglobulin.

### 6. Storage

Before storage, it is recommended to wash the cartridge with at least 5 ml of 20% ethanol.

Keep at +2°C - +8°C. Do not freeze.

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