ANTIBODY PURIFICATION

PROCEDURE FOR USE
Protein G Affinity Cartridges (5ml)

DESCRIPTION

Protein G Affinity Cartridges 5ml are used for purification of classes, subclasses and fragments of immunoglobulins from cell culture media and biological fluids. Protein G is immobilized by means of covalent binding that avoids protein loss and allows for column re-use.

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

Cartridges (5 ml) can be operated with liquid chromatography systems (such us ÄKTA™ design systems) via standard 10-32 fittings without additional connectors. The recommended flow rate is 5 ml/min.

1. Elimination of the Preservative

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 cartridge with 5 - 10 column volumes of distilled water to eliminate the preservative.

2. Equilibration of the Cartridge

Equilibrate the cartridge with 5 - 10 column volumes of binding buffer at the temperature at which the purification will be performed.

Binding buffer: IgG from most species binds at neutral pH. The buffers used most frequently are sodium phosphate (25 mM) pH 7.0.

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.

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4. Washing of the Cartridge

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 – 2.5.

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.

6. Storage

Keep at +2ºC - +8ºC in a suitable bacteriostat, e.g. 20% ethanol. Do not freeze.