Protein A/G AGAROSE BEADS
PROCEDURE FOR USE
Bulk Resins

DESCRIPTION

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/G Agarose Resin 4 Rapid Run™ contains a mixture of 50% Protein G Agarose Resin 4 Rapid Run™ & Protein A Agarose Resin 4 Rapid Run™ in 20% ethanol. This resin is used to isolate mouse IgG_1, IgG_{2a}, IgG_{2b}, IgG_3 and IgA, rat IgG_1, IgG_{2a}, IgG_{2b}, IgG_{2c}, rabbit and goat polyclonal and human IgG_1, IgG_2, IgG_3 and IgG_4.

Protein G and Protein A are immobilized by means of covalent binding that avoids protein loss and allows for column re-use.

This product is supplied as a suspension in 20% ethanol 50:50 (Mixture of Resins: Preservative).
Static Binding Capacity: ~ 25 mg human IgG / ml resin
Resin: 4% highly crosslinked agarose beads

INSTRUCTIONS

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 resin

Equilibrate the column 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.

4. Washing of the 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 – 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.

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6. Storage

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

COLUMN PACKAGING

1. Gently shake the resin bottle several times to obtain a homogeneous suspension of Protein A/G Agarose Resin 4 RAPID RUN™ 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 1cm 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 - 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.

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/G.	- Re-pack column.

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	Column has not been stored in recommended conditions alter previous usage.	- Always follow manufacturer recommendations.
	The antibody to be purified has low affinity with Protein A/G.	- 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 4ºC) 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.

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