Ig-Thio-Capture Beads

Cat. 1015-5/10/25

Multi-species immunoglobulins purification support

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Introduction

Ig-Thio-Capture Beads absorbent is a chemical based immunoadsorbent aimed for the purification of immunoglobulins. **Ig-Thio-Capture Beads** support is based on the ability of some proteins, especially immunoglobulins, to bind to an immobilized chemical ligand that contains a sulfone group in close proximity to a thioether. The **Ig-Thio-Capture Beads** also termed thiophilic adsorption chromatography provides a low cost, efficient alternative to other antibody purification methods. The purification with thiophilic gel is a simple, rapid, one step purification method for antibody purification from serum, ascites or tissue culture supernatant. In addition, the method exhibits good protein recovery with excellent preservation of antibody activity due to gentle elution conditions, which usually yield concentrated, essentially salt-free, highly purified immunoglobulins at near neutral pH. The gel has a high binding capacity and it has a broad specificity toward immunoglobulins derived from various animal species irrespective of the type of immunoglobulin or the immunoglobulin subclass.

Ig-Thio-Capture Beads characteristics.

Matrix: Sepharose CL-4B
Activation method: divinyl sulfone.
Chelating group: sulfone group in proximity to a thioether group
Binding capacity: ~8-12 mg IgG per ml of beads
Mean bead size: 40-165 µm
Bead structure: Highly cross-linked spherical agarose, 6%
Max back pressure: 0.3 MPa, 3 bar
Max. flow rates: 4 ml/min/cm2
Recommended flow rate: 1-3 ml/min/cm^2
Stability of the matrix: pH 3-13.
Storage: 4°C in PBS pH 7.4 added with NaN_3 0.1% (w/v) as a preservative.

Protocol: Multi-species immunoglobulins purification

A. Buffers needed

Prepare the following solutions (the final volume to be prepared depend on the final bed volume of Ab-Gel)

Binding buffer – 0.5 M potassium sulfate, 50 mM sodium phosphate pH 8.0

Elution buffer - 50 mM sodium phosphate pH 8.0

Storage buffer – 0.5 M Tris and 0.05% sodium azide pH 7.4

Regeneration solution – 8 M guanidine hydrochloride

B. Sample Preparation

While mixing, add 87 mg of potassium sulfate (K_2SO_4) into 1 ml of sample to be purified (serum or crude antibody sample). Mix the sample gently to avoid denaturation of the immunoglobulin. When the salt is fully dissolved, centrifuge the sample at 10,000 x g for 10 min. Carefully remove the clear supernatant and save for the next purification steps.

C. Packing thiophilic gel into column

1. Allow the thiophilic gel slurry (50% slurry) to come to room temperature.
2. Mix the slurry thoroughly until complete disappearance of the gel precipitate. Add the mixed suspension into an empty column and allow the gel to settle in the column.
3. Wash the column with 5-6 bed volume of DDW.

D. Purifying Immunoglobulins

1 Equlibrate the column with 5 column volumes of Binding buffer (0.5 M potassium sulfate, 50 mM sodium phosphate pH 8.0).

2 Apply the sample to the column and allow the sample to completely enter the gel.

3 Wash the column with 12 column volumes of Binding buffer. Monitor the absorbance of the fraction at 280 nm (alternatively quantified protein eluted by Bradford assay) to determine when all non-bound material is washed from the column.

4 Elute the column with 6-12 column volumes of Elution buffer and continue to collect the column effluent bound fractions. Measure the absorbance (alternatively quantified protein eluted by Bradford assay) of each bound fraction. The second, third and fourth bound fractions will usually contain the highest concentration of purified immunoglobulins.

D. Re-equilibration and Storage

1. Re-equilibration the column by washing it with 10 column volumes of Regeneration buffer.
2. Wash the column with 10 volumes of distilled water.
3. Equilibrate the column with the Binding buffer.
4. Storage conditions: equilibrate the column with the Binding buffer added with NaN_3 0.1% (w/v) as preservatives.