ADAR BIOTECH

Protein A Beads

Cat. 1016-1/5/10/25

Immunoglobulins affinity-purification beads ver. 1.2

Introduction

The origin of protein A is from the bacteria Staphylococcus aureus. Protein A is capable of binding most classes and subclasses of immunoglobulins from various species. **Adar's Protein A beads** are produced using genetically engineered form of Protein-A. Non-essential regions have been removed while leaving the IgG binding sites intact. The recombinant Protein A has been immobilized to Sepharose beads, in order to create a winner product for the purification of various mammalian immunoglobulins.

Protein A beads are being routinely used for the isolation of IgG from several species of mammals. The binding of Protein A to IgGs vary between animal species and between IgG subclasses within the same species. Major limitation of protein A lies with the weak binding it presents towards mouse IgG1-a common IgG subclass. Despite that, Protein A possesses useful properties that make it a popular choice for the isolation of most types of IgGs. Protein A binds IgGs through the F_c region of the molecules leaving the F_{ab} region available for binding the antigen. **Protein A Beads** are commomnly used for antibodies purification and for the isolation of immune complexes by Immunoprecipitation (IP).

Protein A Beads Specifications

Matrix: Sepharose™ CL-4B
Protein A density: 2-4.5 mg/ml of resin
Binding capacity: ~4-8 mg rabbit IgG per ml of resin
Mean bead size: 40 -165 µm
Bead structure: Highly cross-linked spherical agarose, 4%
Max back pressure: 0.3 MPa, 3 bar
Max. flow rate: 4 ml/min/cm^2
Recommended flow rate: 1-3 ml/min/cm^2
Stability of the matrix: pH 2-11.
Storage: 4°C in PBS pH 7.4 added with NaN_3 0.1% (w/v) as a preservative.

Protocol: Immunoglobulins affinity-purification using Protein A Beads

A. Buffers required

Binding buffer (1L): Phosphate buffered saline (PBS) pH 7.4

Neutralization buffer (50ml): Tris 1M pH 8.0 Adjust pH to 8.0 by 1M HCl.
Wash buffer (200 ml): PBS pH 7.4 plus 0.05% Tween 20 .
Elution buffer (100ml).: 0.1M glycine buffer Adjust pH to 2.8 by acetic acid.

Storage buffer: PBS pH 7.4 added with NaN_3 0.1% (w/v) as a preservative.

B. Sample preparation

Remark: work at 0°C to at 4°C in order to minimize antibodies degradation whenever possible.

1. If serum is the source dilute 5 ml to 25 ml final volume with Binding buffer at 4°C.
2. Centrifuge diluted serum supernatants for 15 minutes at 4000xg at 4°C to sediment debris.
3. Filter supernatants through 0.45u filter.

C. affinity-purification

Remark: Typically 5 ml of bed volume are used to purify antibodies from 0.5L of monoclonal antibody-containing culture, or 25 ml of x5 diluted serum.

1. Wash Protein A beads with 30 ml Binding buffer three times in 50 ml conical tube. Allow the beads to sediment naturally for 5 minutes each time, and remove upper liquid phase with a pipette.
2. Use the washed beads to pack a 1-1.5 cm diameter column by pouring the beads into an empty column. After column preparation equilibrate the column with Binding buffer by washing with 5-10 column volumes. Recommended flow rates are 1-2 ml/min/cm^2.
4. Bring the sample to room temperature. Apply the sample to column at a rate between 0.1 ml/min to 0.2 ml/min, using a syringe or a pump. The total volume of the sample applied is not critical in most cases. Save the flow through fraction for SDS-PAGE analysis.
5. Wash with 5-10 column volumes of Wash buffer.
6. Prepare labeled microfuge tubes with 5% V/V of neutralization buffer in each tube. Place tubes on an ice bucket
7. Elute with Elution buffer into the labeled microfuge tubes, at flow rates of 1-2 ml/min/cm^2. Two to five column volumes are usually needed for elution of the immunoglobulins. Use the elution buffer as blank when doing the quantitation of the target protein in eluted fractions.

D. Re-equilibration and Storage

1. Immediately at the end of elution step, wash column with 10 bed volumes of Binding buffer.
2. Store Storage conditions: Store column in a refrigerator with Storage buffer.

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Protocol: Immunoprecipitation (IP) using Adar's Protein A or Protein G Beads

Reagents

- Adar's Protein A (or protein G) Beads (100-200 µl 50% slurry per sample)
- Primary antibody (about 5µg per sample)
- Immunoprecipitation buffer (RIPA or PBS). RIPA: 30 mM sodium phosphate, pH 7.4, 500 mM NaCl, 0.5% sodium deoxycholate, 0.1% SDS, 1% NP40. PBS: 50mM phosphate buffered Saline with 1% Triton TX-100
- Elution Buffer: 0.1 M glycine, pH adjusted to 2.6 with HCl.
- Protease inhibitor cocktail (commercially available from several vendors)

Procedure for Immunoprecipitation of cellular antigens

1. Wash 10^7 mammalian cells twice with cold PBS. Centrifuge after each wash at 1000 RPM.
2. Count cells using a hemocytometer. Add 1 ml ice-cold PBS added with 1% Triton TX-100 and protease inhibition cocktail. Gently rock the suspension on a shaker at 4˚C for 15 minutes to lyse cells.
3. Centrifuge the lysate at full speed in a precooled microfuge for 10 minutes. Immediately transfer the supernatant to a fresh centrifuge tube and discard the pellet.
4. To prepare protein A or G agarose/sepharose for work, wash the beads twice with PBS and restore to a 50% slurry with fresh PBS. Cut the pipette tips to allow free work with beads.
5. Pre-clear the cell lysate (recommended step) by adding 100 µl of either protein A or G agarose/sepharose bead slurry (50%) per 1 ml of cell lysate and incubating at 4°C for 10 minutes on a shaker. Pre-clearing the lysate will reduce non-specific binding of proteins to the beads when used later on in the assay.
6. Remove the protein A or G beads by centrifugation at 14,000 x g at 4°C for 10 minutes. Transfer the supernatant to a fresh centrifuge tube and discard beads.
7. Determine the protein concentration of the cell lysate, e.g. by performing a Bradford assay. Dilute a sample taken out of the cell lysate at least 1:10 before determining the protein concentration because of the interference of the detergents in the lysis buffer with the Bradford reagent.
8. Dilute the cell lysate with PBS to approximately 1 mg/ml total protein concentration.
9. If you work with difference source of antigen rather than cells lysate you might as well use the RIPA buffer that will solubilize more types of antigens due to higher content of detergents.
10. To 100 µl antigen-containing solution at approximately 1mg/ml total protein content, add a 2 to 4 fold molar excess of primary antibody to the protein solution containing the antigen of interest. About 3-5 µg of purified antibody would be a good starting point in

case of doubt. Adjust the volume of the sample to 0.2 ml with immunoprecipitation buffer (RIPA or PBS).

11. Incubate the sample 2 hours at room temp. with occasional mixing or overnight at 4°C.
12. Add appropriate amount of Adar's protein A beads or Adar's protein G beads to the antigen-antibody complex (~ 50 µl of 50% beads slurry or 25 µl of net bead volume per 5 µg of antibody). Cut pipettor tips to allow work with beads-containing solution.
13. Incubate the sample with gentle mixing for 2 hours at room temperature.
14. Wash the immobilized protein A or G-bound complexes with 0.5 ml of the immunoprecipitation buffer (RIPA or PBS), followed by centrifugation for 2-3 minutes in a microcentrifuge at low speed (1,000-2,000 RPM) to preserve beads shape. Discard the supernatant. Repeat this wash procedure at least 3 times.
15. Adjust volume of washed beads to 40ul with fresh immunoprecipitation buffer (RIPA or PBS). Add 10 µl reducing gel loading dye and incubate the beads for 5 minutes at 95°C. Cool on ice for 10 seconds, spin down in microfuge at full speed for 30 seconds
16. Load immediately about 10 µl on SDS-PAGE for analysis.