Anti- Rabbit Beads

Cat. 1031-5/10/25

Immunoprecipitation Beads

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Introduction

Goat Anti-Rabbit IgG (whole molecule) was affinity purified on Rabbit IgG affinity column using IgG isolated from pooled normal Rabbit sera. Eluted antibodies were further purified by Protein G column in order to separate IgGs from other classes of antibodies. Purified Antibodies were coupled to Sepharose Beads using TargetLock Chemistry.

Anti-Rabbit Beads Specifications

Matrix: Sepharose™ CL-4B
Coupling method: TargetLock.
Coupling density: 0.7-1.2 mg/ml of Goat anti Rabbit IgG
Rabbit IgG binding capacity: minimum 0.6 mg/ml of beads
Mean bead size: 40 -165 µm
Range of pH values allowed: 3.0-11.0
Bead structure: Highly cross-linked spherical agarose, 4%
Max back pressure: 0.3 MPa, 3 bar
Max. flow rates: 4 ml/min/cm^2
Recommended flow rate: 1-3 ml/min/cm^2
Storage: 4°C in PBS pH 7.4, 0.3N NaCl added with 01% (w/v) Azide as a preservative.

Protocol: Immunoglobulins affinity-purification using Anti-Rabbit 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 optional PBS plus 1% Triton X-100.
Elution buffer (100ml): 0.1M glycine buffer Adjust pH to 2.8 by 1N HCl.
Regeneration buffer (100ml): 0.1M glycine buffer Adjust pH to 2.4 by 1N HCl.
Storage buffer: PBS with 0.1% sodium azide 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 15ml final volume with Binding buffer at 4°C.
2. Centrifuge diluted serum supernatants for 15 minutes at 10,000xg at 4°C to sediment debris.
3. Filter supernatants through 0.45µm filter.

C. Column affinity-purification

Remark: Typically 5 ml of bed volume are used to purify antibodies from 0.5L of monoclonal antibody-containing culture, or 50 ml of x10 diluted serum.

1. Wash protein Anti-Rabbit 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.
3. Bring the sample to room temperature. Apply the sample to column at a rate between 0.2 ml/min to 0.5 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.
4. Wash with 20 column volumes of Wash buffer.
5. Prepare labeled microfuge tubes with 5% V/V of neutralization buffer in each tube. Place tubes on an ice bucket
6. Elute with Elution buffer into the labeled microfuge tubes, at flow rates of 1-2 ml/min/cm^2. Two to four 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, Regeneration and Storage

1. Regenerate column by 10 column volumes of the regeneration buffer. Work quickly as exposure of column to pH 2.4 should be kept to a minimum.
2. Wash column with 10 bed volumes of Binding buffer, immediately at the end of elution step.
3. Storage conditions: Store column in a refrigerator with Storage buffer.

Protocol: Immunoprecipitation (IP) using Anti-Rabbit Beads

Reagents

- Adar's or Anti-Rabbit 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 added 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 Anti-Rabbit Beads 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 Anti-Rabbit Beads 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 Anti-Rabbit 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 Anti-Rabbit 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 Anti-Rabbit Beads 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.