ProteoChem
Crosslinking Protein Biology & Chemistry

Protocol for Crosslinking Antibody to Immobilized Protein A or Protein G Resin

Procedure for BS3 Crosslinking Antibody to Immobilized Protein A or Protein G

1. Load antibody onto resin using resin manufacturer's protocol. Ensure antibody binding/wash buffer do not contain primary amines such as glycine or Tris. If antibody is stored in an amine-containing buffer, then remove amines by buffer exchange (dialysis or resin filtration) into PBS (i.e. 100 mM Sodium Phosphate, 150 mM NaCl, pH 7.2) prior to loading antibody onto the resin.

   NOTE: Amine buffers are not compatible with the crosslinker BS3.

Crosslinking the Protein A or Protein G Bound Antibody

1. For every 2 ml of immobilized Protein A or Protein G resin used dissolve 15 mg BS3 crosslinker in 2 ml PBS (100 mM Sodium Phosphate, 150 mM NaCl, pH 7.2).

   NOTE: BS3 is moisture-sensitive, and to avoid moisture condensation onto BS3 the vial must be equilibrated to room temperature before opening.
2. Immediately add the crosslinking solution to the antibody-bound resin.
3. Ensure resin is in a sealed column or tube and suspend resin by repeated inversion for 60-90 minutes at room temperature.
4. Upon completion of the reaction, transfer the slurry to a column (if not already in one) and allow the resin to settle. Then let the residual solution passes through the column. Don't let the resin bed to dry.
5. Wash the crosslinked resin with 5 resin volumes of PBS and replace bottom cap when wash solution reaches the level of the resin bed.

Block Unreacted Crosslinker

1. Add 2 ml of 100 mM Ethanolamine, pH 8.2 Blocking Buffer to column to block any non-reacted NHS-ester crosslinker groups.
2. Ensure resin is in a sealed column or tube and suspend resin by continued inversion for 10-15 minutes at room temperature.
3. Upon completion of the blocking reaction, transfer the slurry to a column (if not already in one) and allow the resin to settle. Then let the residual solution passes through the column. Don't let the resin bed dry out.
4. Seal bottom of column and add 6 ml of 100 mM Glycine-HCl, pH 2.8 IgG Elution Buffer. Seal top of column and continually invert column to completely mix the resin.
5. Allow the resin to settle. Then let the residual solution passes through the column. Don't let the resin bed dry out. Wash column with an additional 5 ml of 100 mM Glycine-HCl, pH 2.8 IgG Elution Buffer.

   NOTE: Elution Buffer will elute non-covalently bound IgG attached to Protein A or Protein G. The antibody concentration of the flow-though may be assayed to determine crosslinking reaction efficiency.
6. Wash the resin with 5 resin volumes of PBS.
7. The affinity column may be stored in an aqueous solution (i.e., Tris or phosphate buffer) containing 0.02% Sodium Azide.

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General Protocol for Affinity Purification of an Antigen

NOTE: This is a general protocol for 2 ml gravity flow column, but since some antigens require more or less stringent conditions for dissociation from an immobilized antibody, this protocol may require optimization.

1. Allow the prepared affinity resin to equilibrate to room temperature.
2. Remove top cap, then the bottom cap and allow storage solution to drain. Do not allow the resin to dry.
3. Equilibrate column with 5 column volumes of PBS Binding Buffer (100 mM Sodium Phosphate, 150 mM NaCl, pH 7.2).
4. Dilute antigen sample at least 1:1 with PBS Binding Buffer.
5. Add sample to the affinity column and incubate at room temperature for 1-2 hours OR overnight at 4°C.
6. Wash the column with PBS Binding Buffer until baseline absorbance at 280 nm is maintained.
8. Elute with 100 mM Glycine-HCl, pH 2.8 IgG Elution Buffer.
7. Collect 1 ml fractions and check protein concentration by measuring absorbance at 280 nm.
8. Adjust the pH of the eluted fractions to neutral with an appropriate concentrated buffer (i.e. 1 M Tris-HCl, pH 9.5; use approximately 0.05 ml per ml of fraction collected).

Column Regeneration

1. Wash with 5 column volumes of 100 mM Glycine-HCl, pH 2.8 IgG Elution Buffer.
2. The affinity column may be stored in an aqueous solution (i.e., Tris or phosphate buffer) containing 0.02% Sodium Azide.

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