Amino-Reactive Resin Protocol and Product Information Sheet

Product Category:	Immobilization Resins
Catalog Number(s):	g4102-15ml, g4102-50ml, g4102-250ml
Product Name:	Amino-Reactive Resin

Amino-Reactive Resin

Amino-Reactive Resin 15 ml (g4102-15ml), 50 ml (g4102-50ml), or 250 ml (g4102-250ml) of settled gel is supplied as 50% slurry in buffer containing 0.02% sodium azide as a preservative.

Gel Support: Cross-linked 6% beaded agarose resin.

Storage: Upon receipt store at 4°C (shipped at ambient temperature).

Procedure for Amino-Reactive Resin Ligand Coupling

Note: The following protocol must be optimized for each specific application

A. Protein Sample Preparation

1. Prepare coupling buffer consisting of 100 mM Sodium phosphate, 150 mM Sodium Chloride, pH 7 (or other suitable buffer). DO NOT use buffers containing primary amines (i.e. Tris, Glycine, etc.).
2. Dissolve or dilute protein to 1-15 mg/ml in 1 ml coupling buffer. If protein is already in solution, then dialyze or desalt into the coupling buffer. You may save 100 ul for determining coupling efficiency in step B.3.

B. *Protein Immobilization Reaction*

1. Wash 2-3 ml Amino-Reactive Resin with 5-10 resin volumes of coupling buffer.
2. Combine oxidized glycoprotein and washed Amino-Reactive Resin in a 15 ml tube. Add 10-15 mg Sodium cyanoborohydride ($NaCNBH_3$; product cr8112-1gm) per ml resin (~50 mM $NaCNBH_3$) while in a fume hood. **Caution:** $NaCNBH_3$ is toxic! Cap tube and mix 6 hours to overnight with gentle end over end rotation.
3. Remove the cap in the hood and drain the contents to a new collection tube. The flow through can be saved to determine the coupling efficiency when comparing the protein concentration to the unbound fraction from step A.2.
4. Wash the resin with 5-10 resin volumes of coupling buffer.
5. Wash the resin with 5-10 resin volumes of 1 M NaCl.
6. Wash the resin with 5-10 resin volumes of coupling buffer.
7. Store in 0.02% sodium azide containing buffer and store at 4°C or immediately proceed to step C.

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C. *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.
1. 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).

D. *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.

References:

Hermanson, et al., (1992) Immobilized Affinity Ligand Techniques. Academic Press, San Diego, CA.

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