Carboxyl-Reactive Resin Protocol and Product Information Sheet

Product Category:	Immobilization Resins
Catalog Number(s):	g4104-15ml, g4104-50ml, g4104-250ml
Product Name:	Carboxyl-Reactive Resin (Immobilized DADPA)

Carboxyl-Reactive Resin

Carboxyl-Reactive Resin 15 ml (g4104-15ml), 50 ml (g4104-50ml), or 250 ml (g4104-250ml) 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.

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

Procedure for Carboxyl-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 100mM MES (cr8107), 0.9% Sodium Chloride, pH 4.5 (or other suitable buffer).
2. Dissolve or dilute protein to 1-10 mg/ml in 2ml coupling buffer. If protein is already in solution, then dialyze or desalt into the coupling buffer. You may save 100ul for determining coupling efficiency in step B.3.

B. *Protein Immobilization Reaction*

1. Wash 2-3 ml Carboxyl-Reactive Resin with 5-10 resin volumes of coupling buffer.
2. Combine protein and washed Carboxyl-Reactive Resin in a 15 ml tube. Add 30 mg EDC-HCl (c1100) per ml resin while in a fume hood. Cap tube and mix 3-4 hours with gentle end over end rotation.
3. Remove the cap 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 1M NaCl.
6. Wash the resin with 5-10 resin volumes of coupling buffer.
7. Store in 0.02% sodium azide containing PBS, or other suitable buffer, and store at 4°C or immediately proceed to step C.

ProteoChem * 5570 W. 290 N. Suite 2 * Hurricane, UT 84737
Toll Free: 888.501.2436 * Phone: +1.720.239.1630 * Fax: +1.720.239.1631
Technical Support E-mail: ts@proteochem.com
www.proteochem.com

C. *General Protocol for Affinity Purification of an Antigen*

NOTE: This is a general protocol for 2ml 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. **Caution:** Do not allow resin bed to dry or crack.
3. Equilibrate column with 5 column volumes of PBS Binding Buffer (100mM Sodium Phosphate, 150mM 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 100mM Glycine-HCl, pH 2.8 IgG Elution Buffer.
7. Collect 1ml fractions and check protein concentration by measuring absorbance at 280nm.
8. Adjust the pH of the eluted fractions to neutral with an appropriate concentrated buffer (i.e. 1M Tris-HCl, pH 9.5; use approximately 0.05ml per ml of fraction collected).

D. *Column Regeneration*

1. Wash with 5 column volumes of 100mM 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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