Carbohydrate-Reactive Resin Protocol and Product Information Sheet

Product Category:	Immobilization Resins
Catalog Number(s):	g4103-15ml, g4103-50ml, g4103-250ml
Product Name:	Carbohydrate-Reactive Resin

Carbohydrate-Reactive Resin

Carbohydrate-Reactive Resin 15 ml (g4103-15ml), 50 ml (g4103-50ml), or 250 ml (g4103-250ml) 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 Carbohydrate-Reactive Resin Ligand Coupling

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

A. Glycoprotein Oxidation

1. Prepare coupling buffer consisting of 100mM Sodium phosphate, pH 7 (or other suitable buffer).
2. Dissolve or dilute protein to 1-10mg/ml in 1ml coupling buffer.
3. Add 5mg Sodium meta-Periodate (cr8103-5gm) to an amber vial and add glycoprotein sample. Mix 30 minutes in the dark (Note: Reaction is light sensitive).
4. Halt reaction by buffer exchange with a 5ml Sephadex G-25 (or equivalent) desalting column. Equilibrate desalting column with coupling buffer. Pass the oxidized sample into the column and elute with coupling buffer. Collect eluent fractions (~2ml) and monitor oxidized protein containing fractions by absorbance at 280nm.

B. *Glycoprotein Immobilization Reaction*

1. Wash 2-3ml Carbohydrate-Reactive Resin with 5-10 resin volumes of coupling buffer.
2. Combine oxidized glycoprotein and washed Carbohydrate-Reactive Resin in a 15ml tube. Cap tube and mix 6 hours to overnight with gentle end or end rotation.
3. Wash the resin with 5-10 resin volumes of coupling buffer and store in 0.02% sodium azide containing buffer and store at 4°C or immediately proceed to step C.

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.

ProteoChem, Inc. * 7300 Edward Dr. * Loves Park, IL 61111
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

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 (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 280nm is maintained.
1. Elute with 100mM Glycine-HCl, pH 2.8 IgG Elution Buffer.
7. Collect 1 ml 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.

ProteoChem, Inc. * 7300 Edward Dr. * Loves Park, IL 61111
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