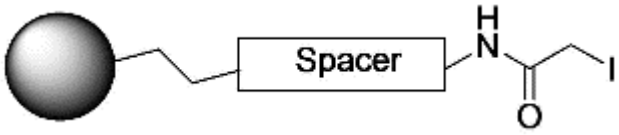
ProteoChem
Crosslinking Protein Biology & Chemistry

Sulfhydryl-Reactive Resin Product Information Sheet and General Protocol

Product Category:	Immobilization Resins
Catalog Number(s):	g4101-15ml, g4101-50ml, g4101-250ml
Product Name:	Sulfhydryl-Reactive Resin



Sulfhydryl-Reactive Resin

Sulfhydryl-Reactive Resin 15 ml (g4101-15ml), 50 ml (g4101-50ml), or 250 ml (g4101-250ml) of settled gel is supplied as a 50% slurry in buffer containing 50% glycerol, 10 mM EDTA, and 0.02% sodium azide as a preservative.

Gel Support: Cross-linked 6% beaded agarose.

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

Immobilization of Sulfhydryl Containing Peptides or Protein

I. Additional Necessary Materials
   a. Use plastic or glass column suitable for the volume of Sulfhydryl-Reactive Resin used
   b. 50 mM Tris, 5 mM EDTA, pH 8.5 as Coupling Buffer (20 times the volume of Sulfhydryl-Reactive Resin used is needed)
   c. 50 mM L-cysteine•HCl in Coupling Buffer as Quenching Buffer
   d. 1 M NaCl as Wash Solution
   e. Phosphate-buffered saline (PBS) containing 0.05% sodium azide as Storage Buffer

II. Equilibrate Sulfhydryl-Reactive Resin Column
   a. Allow Sulfhydryl-Reactive Resin and all reagents to reach room temperature
   b. Mix Sulfhydryl-Reactive Resin bottle to uniformly resuspend the resin as a slurry
   c. Transfer the correct volume of 50% resin slurry to an unfilled column using a wide bore pipette tip (i.e. aliquot 2 ml of resin slurry to achieve a 1 ml resin bed volume)
   d. Equilibrate your column with 4 bed volumes of Coupling Buffer, but do not allow the resin bed to become dry during equilibration
   e. Stop the flow of Coupling Buffer or add more buffer solution whenever the buffer reaches the top of the resin bed

III. Conjugate Protein or Peptide to Sulfhydryl-Reactive Resin
   a. Suspend reduced protein or peptide in Coupling Buffer

b. Add 1-2 ml of protein or peptide solution per milliliter of Sulfhydryl-Reactive Resin to the column (optional: save retain of protein/peptide solution to determine coupling efficiency in step g)
c. Cap and gently rock to mix column at room temperature for 15-20 minutes
d. Set the column in an upright position and allow the resin to incubate at room temperature without mixing for an additional 30 minutes
e. Remove top then bottom column caps and let the solution drain from the column into a clean collection tube
f. Position the column over a new collection tube and wash the resin with 3 resin-bed volumes of Coupling Buffer
g. Optional: Calculate the conjugation effectiveness by comparing the protein or peptide concentrations of the unconjugated fraction (step e) to the starting sample (step b)

IV. Block Unbound Binding Sites on Sulfhydryl-Reactive Resin

a. Cap the bottom of the column
b. Add one resin bed volume of Quenching Buffer (50 mM L-Cysteine-HCl in Coupling Buffer)
c. Cap the top of the column and mix Sulfhydryl-Reactive Resin 15-20 minutes at room temperature, then incubate the quenching reaction without mixing for an additional 30 minutes
d. Remove the top cap, then the bottom cap and remove the column's Quenching Buffer
e. Wash column with at least 5-10 resin bed volumes of 1 M NaCl Wash Solution
f. Wash column with 2 resin bed volumes of Storage Buffer
g. Cap the bottom of the column and add one resin bed volume of Storage Buffer
h. The resin filled column can now be capped and stored at 4°C or used for affinity purification

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.

a. Allow the prepared affinity resin to equilibrate to room temperature.
b. Remove top cap, then the bottom cap and allow storage solution to drain. Do not allow the resin to dry.
c. Equilibrate column with 5 column volumes of PBS Binding Buffer (100 mM Sodium Phosphate, 150 mM NaCl, pH 7.2).
d. Dilute antigen sample at least 1:1 with PBS Binding Buffer.
e. Add sample to the affinity column and incubate at room temperature for 1-2 hours OR overnight at 4°C.
f. Wash the column with PBS Binding Buffer until a baseline absorbance at 280 nm is maintained.
g. Elute with 100 mM Glycine-HCl, pH 2.8 IgG Elution Buffer.
h. Collect 1 ml fractions and check protein concentration by measuring absorbance at 280 nm.
i. 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).

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