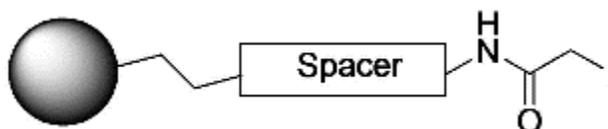


Sulfhydryl-Reactive Resin Product Information Sheet and General Protocol

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. Sulfhydryl-Reactive Resin binding capacity is 0.5-1 mg peptide or 1-10 mg protein per ml resin.

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 [1-10 mg/ml] or peptide [0.05-0.5 mg/ml] 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).