INSTRUCTIONS

SulfoLink® Coupling Resin

20401 20402 20404

0527.3

Number	Description
20401	**SulfoLink Coupling Resin**, 10mL settled resin (20mL resin slurry)
20402	**SulfoLink Coupling Resin**, 50mL settled resin (100mL resin slurry)
20404	**SulfoLink Coupling Resin**, 250mL settled resin (500mL resin slurry)
	Support: 6% crosslinked beaded agarose
	Supplied as a 50% slurry in storage buffer (10mM EDTA-Na, 0.05% NaN_3, 50% glycerol)
	Storage: Upon receipt store product in the dark at 4°C. Product is shipped at ambient temperature.

Introduction

The Thermo Scientific SulfoLink Coupling Resin allows covalent immobilization of sulfhydryl-containing peptides, proteins and other ligands to a beaded agarose support for use in affinity purification procedures. Iodoacetyl groups on the SulfoLink Coupling Resin react specifically with free sulfhydryls (Figure 1). The 12-atom spacer arm minimizes steric hindrance, ensuring efficient binding interactions with the coupled molecule. The resin is ideal for conjugating peptides for antibody purification and can immobilize approximately one milligram sulfhydryl-containing peptide per milliliter of settled resin.

The SulfoLink Coupling Resin also can be used as an immobilization support for proteins and other molecules. For example, greater than five milligrams of reduced human IgG can be immobilized per milliliter of settled resin. Any molecule that contains free (reduced) sulfhydryl groups can be immobilized on SulfoLink Coupling Resin. Reducing agents will cleave disulfide bonds to make sulfhydryl groups available for coupling; however, sulfhydryl-containing reducing agents will compete for immobilization and must be removed by gel filtration before the coupling reaction. Depending on the size of the ligand to be immobilized, other methods for sample preparation may be needed (see Important Procedural Notes).

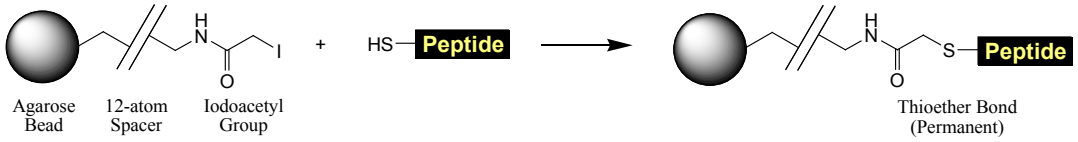

Figure 1. General structure and reaction scheme for the Thermo Scientific SulfoLink Coupling Resin.

Important Procedural Notes

The peptide or protein to be immobilized must have free (reduced) sulfhydryls. Ellman's Reagent (Product No. 22582) may be used to determine if the peptide or protein contains free sulfhydryls. To make sulfhydryl groups available for coupling, cleave disulfide bonds with a reducing agent). If a sulfhydryl-containing reducing agent was used, desalting or dialysis must be performed to remove the reducing agent before immobilization.

- **Peptide samples:** Tris(2-carboxyethyl)phosphine (TCEP, Product No. 77720), efficiently reduces peptides but does not interfere with iodoacetyl coupling, requiring no removal of excess reagent before immobilization. TCEP is stable in aqueous solution and selectively reduces disulfide bonds.

  Dissolve or dilute 0.1-1mg of peptide in 2mL of Coupling Buffer and add TCEP to a final concentration of 25mM TCEP. Note that TCEP interferes with the Thermo Scientific Pierce BCA Protein Assay.

- **Antibody samples:** Reduce disulfide bonds using 2-mercaptoethylamine•HCl (2-MEA, Product No. 20408). 2-MEA can selectively cleave hinge-region disulfide bonds between IgG heavy chains while preserving the disulfide bonds between the heavy and light chains. The result is two half antibodies with sulfhydryls available for immobilization.

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Dissolve or dilute 1-10mg of protein with 1mL of buffer (0.1M sodium phosphate, 5mM EDTA-Na; pH 6.0). Add the protein solution to 6mg of 2-MEA (50mM). Incubate mixture at 37°C for 1.5 hours. Remove 2-MEA by performing two passes through a Thermo Scientific Zeba Spin Desalting Column (see Related Thermo Scientific Products) using the Coupling Buffer.

Procedure for Immobilizing a Peptide or Protein Having Free Sulfhydryls

A. Additional Materials Required

- Column: Choose a glass or plastic column size appropriate for the volume of SulfoLink Resin to be used. The Disposable Column Trial Pack (Product No. 29925) contains accessories plus two each of three different column sizes, appropriate for 0.5-10mL resin bed volumes. Alternatively, several centrifuge-ready Thermo Scientific Pierce Columns are available for resin bed volumes from 25 µl to 10mL.
- Coupling Buffer: 50mM Tris, 5mM EDTA-Na; pH 8.5. Prepare a volume equal to 20 times the volume of SulfoLink Resin to be used.
- Quenching Reagent: L-cysteine•HCl (Product No. 44889)
- Wash Solution: 1M sodium chloride (NaCl)
- Storage Buffer: Phosphate-buffered saline (PBS) or other suitable buffer containing 0.05% sodium azide (NaN_3)

B. Prepare SulfoLink Resin Column

1. Equilibrate SulfoLink Coupling Resin and all other reagents to room temperature.
2. Stir or swirl bottle to evenly suspend the resin, and then use a wide-bore pipette to transfer an appropriate volume of the 50% resin slurry to an empty column. For example, transfer 2mL of resin slurry to obtain a 1mL resin bed.
3. Equilibrate column with four resin-bed volumes of Coupling Buffer. Replace the bottom column cap.

   Note: When using gravity-flow columns, do not allow the resin bed to become dry at any time throughout the procedure. Add more solution or replace the bottom cap on the column whenever the buffer drains down to the top of the resin bed.

C. Couple Peptide/Protein to Resin

1. Dissolve prepared (i.e., reduced) peptide/protein in Coupling Buffer and add it to the column. Use 1-2mL of peptide or protein solution per milliliter of SulfoLink Coupling Resin. If desired, retain a small amount of the peptide or protein solution for later comparison to the coupling reaction flow-through fraction to estimate coupling efficiency.
2. Replace the top cap and mix column (by rocking or end-over-end mixing) at room temperature for 15 minutes.
3. Stand the column upright and incubate the column at room temperature for an additional 30 minutes without mixing.
4. Sequentially remove top and bottom column caps and allow the solution to drain from the column into a clean tube.
5. Place the column over a new collection tube and wash column with three resin-bed volumes of Coupling Buffer.
6. Determine the coupling efficiency by comparing the protein/peptide concentrations (e.g., by absorbance at 280nm) of the noncoupled fraction (Step 4) to the starting sample (Step 1).

D. Block Nonspecific Binding Sites on Resin

1. Replace the bottom cap on column. Prepare a solution of 50mM L-Cysteine•HCl in Coupling Buffer.
2. Add one resin-bed volume of 50mM cysteine solution to the column. Mix for 15 minutes at room temperature, and incubate the reaction without mixing for an additional 30 minutes.

E. Washing the Column

1. Sequentially remove the top and bottom caps and allow the column to drain.
2. Wash the column with at least six resin-bed volumes of Wash Solution (1M NaCl).
3. Wash the column with two resin-bed volumes of degassed Storage Buffer.

4. Replace the bottom cap and add one additional resin-bed volume of Storage Buffer.
5. The covalently coupled ligand can be used for affinity purification. Replace the top cap and store the column upright at 4°C, or remove the bottom cap and proceed with the General Protocol for Affinity Purification.

General Protocol for Affinity Purification of Protein

Note: This protocol assumes use of a gravity-flow column with a resin-bed volume of 2mL. For different resin-bed volumes, adjust all solution volumes accordingly. The amount of protein sample that can be processed and the binding conditions required depend on the specific affinity interaction used and must be optimized for the particular experiment. Adjust wash and elution buffer volumes to the requirements of the specific purification system.

A. Additional Materials Required

- Binding/Wash Buffer: Use phosphate-buffered saline (PBS; Product No. 29372), Tris-buffered saline (TBS; Product No. 28379) or other buffer that is compatible with the intended affinity interaction. Degas buffers to avoid introducing air bubbles into the resin bed that may impede flow.
- Sample: Dissolve or exchange sample into Binding/Wash Buffer (i.e., a buffer compatible with the affinity interaction).
- Elution Buffer: IgG Elution Buffer (Product No. 21004) or 0.1-0.2M glycine•HCl, pH 2.5-3.0.
- Neutralization Buffer (optional): Prepare 1mL of 1 M sodium phosphate or 1M Tris•HCl (pH 8.5-9.0).

B. Column Preparation

1. Equilibrate the prepared affinity column to room temperature. Remove top and bottom column caps and allow storage solution to drain from column.
2. Add 6mL of Binding/Wash Buffer and allow it to pass through the column.

C. Sample Purification

1. Add Sample and allow it to pass through the column. (Be aware that a 2mL resin bed contains ~1.5mL of solution; as such, the volume of solution retained in the resin bed from the previous step is "pushed out" the bottom by each new 1.5mL of solution that enters the resin bed.)
   - Samples < 2mL: Allow Sample to soak into resin bed, then replace bottom column cap, add 0.2mL of Binding/Wash Buffer, and incubate the column for 1 hour to allow binding to occur. Uncap the column, add 2mL of Binding/Wash Buffer, and collect the emerging nonbound Sample for analysis of column binding efficiency and capacity.
   - Samples > 2mL: Add Sample to column and allow it to pass through into a clean collection tube; if desired, pass the Sample through the column a second time. Alternatively, pass the Sample through the column only once, but replace the bottom cap to stop column flow for 15-30 minutes after each 1.5mL of Sample has entered the resin bed. These techniques ensure sufficient time for binding to occur. If desired, collect and save the Sample flow-through in several fractions for subsequent analysis of column binding efficiency and capacity.
2. Pass 12mL of Binding/Wash Buffer through the column to wash away nonbound components of the Sample solution. (If desired, save the flow-through for analysis of washing efficiency.)
3. Elute protein by passing 8mL of Elution Buffer through the column. Collect separate 1mL fractions, neutralizing each one by adding 50µL of Neutralization Buffer.
4. Determine which elution fractions contain purified protein by checking for those having highest absorbance at 280 nm. Pool fractions of interest and desalt or dialyze into an appropriate buffer for storage or the downstream application.

D. Column Equilibration and Storage

Note: Equilibrate the column soon after use to prevent damage to the immobilized protein by the low pH Elution Buffer. Typically, an affinity column can be reused about 10 times, depending on the stability of the immobilized molecule.

1. Wash and equilibrate the affinity column by passing 16mL of Binding/Wash Buffer through the column.
2. Complete wash step with Binding/Wash Buffer (or other appropriate buffer) that contains 0.05% sodium azide as preservative. Leave 2mL of buffer above resin bed, cap the column and store it upright at 4°C. Do not freeze.

Troubleshooting

Problem	Cause	Solution
Peptide/peptide precipitates in Coupling Buffer	Protein/peptide was not soluble in Coupling Buffer	SulfoLink Coupling Reaction is compatible with up to 20% DMSO and 4M urea – if necessary, add these reagents to the coupling reaction to maintain solubility of the protein/peptide
Low coupling efficiency	Sulfhydryls were not reduced	Reduce protein/peptide and proceed immediately with desalting and coupling procedure to avoid reformation of disulfide bonds
	Sulfhydryl-containing reductant was not removed from sample	Remove reductant from the reduced sample using a desalting column before immobilization
		Reduce peptides using TCEP or Immobilized TCEP Disulfide Reducing Gel (Product No. 77712)
Column flows very slowly	Microscopic air bubbles in column restricted flow	Remove air bubbles by stirring or centrifugation. (See Tech Tip Protocol "Remove Air Bubbles From Columns" on our website)
Affinity column lost binding capacity	Immobilized sample was damaged by time, temperature or elution conditions	Prepare a new affinity column and alter the condition responsible for damaging the column
	Column was fouled with nonspecifically bound material	Prepare a new affinity column and remove foulants from sample before affinity purification

Information Available from our Website

- Tech Tip #7: Remove Air Bubbles from Columns to Restore Flow Rate
- Tech Tip #29: Degas Buffers for use in Affinity and Gel Filtration Columns
- Tech Tip #43: Protein Stability and Storage
- Tech Tip #4: Batch and Spin Cup Methods for Affinity Purification of Proteins

Related Thermo Scientific Products

44999 **SulfoLink Immobilization Kit for Peptides**

44995 **SulfoLink Immobilization Kit for Proteins**

77720 **Bond-Breaker TCEP Solution, Neutral pH,** 5mL

20408 **2-Mercaptoethylamine•HCl (2-MEA)**, 6 × 6mg in amber screw-cap vials

89891 **Zeba Spin Desalting Columns, 5mL,** 5 columns, for 500-2000µL samples

89892 **Zeba Spin Desalting Columns, 5mL,** 25 columns, for 500-2000µL samples

28372 **BupH Phosphate Buffered Saline Pack,** 40 packs

22582 **Ellman's Reagent,** 5g

69700 **Pierce Spin Cup Columns**

29925 **Disposable Column Trial Pack,** 6 columns (two of each size) plus accessories

General References

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Lundblad, R.L. (1991). Chemical Reagents for Protein Modification, 2nd edition, CRC Press: Boca Raton, FL.

Metrione, R.M. (1982). Bromoacetyl Sepharose: A solid phase inhibitor of sulfhydryl enzymes. *Anal Biochem* **120:**91-4.

Wong, S.S. (1991). Chemistry of Protein Conjugation and Crosslinking. CRC Press: Boca Raton, FL, p. 248-51.

Product References

Handlogten, M., *et al.* (2005). Apical ammonia transport by the mouse inner medullary collecting duct cell (mIMCD-3). *Am J Physiol Renal Physiol* **289:**347-58.

Narayan, S. B., *et al.* (2004). CLN3L, a novel protein related to the Batten disease protein, is overexpressed in Cln3-/- mice and in Batten disease. *Brain* **127:**1748-54.

Wilhelmsen, K., *et al.* (2004). Purification and identification of protein-tyrosine kinase-binding proteins using synthetic phosphopeptides as affinity reagents. *Mol Cell Proteomics* **3:**887-95.

Current product instructions are available at *www.thermoscientific.com/pierce*. For a faxed copy, call 800-874-3723 or contact your local distributor.