INSTRUCTIONS

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SulfoLink™ Immobilization Kit for Peptides

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44999

Number	Description
44999	**SulfoLink Immobilization Kit for Peptides,** sufficient reagents to prepare five reusable affinity columns

Kit Contents:

SulfoLink Column, 5 × 2 ml, 6% crosslinked beaded agarose supplied as a 50% slurry in storage buffer (10 mM EDTA-Na, 0.05% NaN_3, 50% glycerol)

Binding Capacity: ~2 mg sulfhydryl-containing peptide

SulfoLink Coupling Buffer, 120 ml, 50 mM Tris, 5 mM EDTA-Na; pH 8.5

Wash Solution, 120 ml, 1.0 M NaCl, 0.05% NaN_3

L-Cysteine•HCl, 100 mg

Bond-Breaker™ TCEP Solution, Neutral pH, 0.5 ml, contains stabilized aqueous 0.5 M TCEP

BupH™ Phosphate Buffered Saline Pack, 1 pack, yields 0.1 M phosphate, 0.15 M sodium chloride; pH 7.2, when reconstituted with 500 ml of water

Column Accessories, porous discs (6), resin separator, and column extender

Storage: Upon receipt store product in the dark at 4°C. Product is shipped at ambient temperature.

Introduction

The SulfoLink Immobilization Kit for Peptides contains all the necessary components for covalent immobilization of sulfhydryl-containing peptides and other ligands to a beaded agarose support. The SulfoLink Coupling Resin is derivatized to contain iodoacetyl groups that react specifically with free sulfhydryls at pH 7.5-9.0 (Figure 1). This kit features a spin-column format that provides fast and easy immobilizations and purifications.

Peptides and other molecules for immobilization must have free (reduced) sulfhydryls. Tris(2-carboxyethyl)phosphine (TCEP), included in this kit, efficiently reduces peptides and other disulfide-containing molecules but does not interfere with iodoacetyl coupling. Phosphines are stable in aqueous solution, selectively reduce disulfide bonds and are essentially nonreactive toward other functional groups commonly present in peptides. TCEP is ideal for peptides and SulfoLink Chemistry because no removal of excess reagent is required before the immobilization reaction.

Once the peptide is immobilized on the SulfoLink Resin, the resulting affinity column can be used for antibody purification. The 12-atom spacer arm minimizes steric hindrance, ensuring efficient antibody-peptide binding interactions. Depending on the stability of the immobilized peptide, the column may be used multiple times without significant loss in binding capacity. The stability of the resin and covalent linkage allow researchers to use buffer conditions that are conducive to forming the desired interactions, making the the SulfoLink Immobilization Kit for Peptides an excellent versatile tool for protein research.

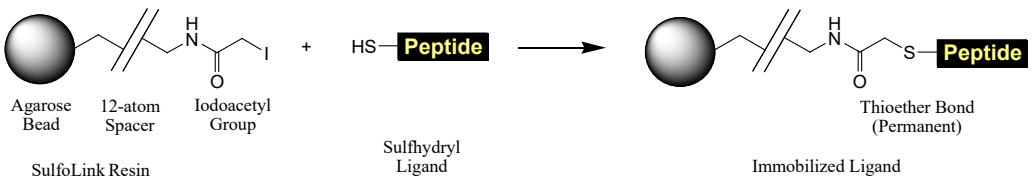

Figure 1. General structure and reaction scheme for the SulfoLink Coupling Resin.

Important Product Information

- Equilibrate kit components to room temperature before processing.
- To quantify the peptide and estimate coupling efficiency use a spectrophotometer or other suitable method. Please note that TCEP interferes with the Pierce™ BCA™ Protein Assay.
- To determine if free sulfhydryls are available on the peptide, use Ellman's Reagent (Product No. 22582) according to the product instructions.
- Hydrophobic peptides may require additional wash steps or addition of detergent during coupling to minimize nonspecific binding.
- Peptides with an isoelectric point (pI) at or near the pH of the Coupling Buffer (pH 8.5) may not solubilize well. Determine the pI for your peptide (*www.embl-heidelberg.de/cgi/pi-wrapper.pl*) before dissolving it in the Coupling Buffer. Additives may assist in peptide solubility if necessary.
- Coupling Buffer pH may be modified only slightly because the reaction requires a degree of ionization of the target functional group. At high pH, undesirable reaction to amino groups can occur. In acidic conditions, reaction to the target sulfhydryls is poor. Therefore, immobilizations are best performed at pH 7.5-9.0.
- Hydrophobic peptides and other ligands may not be readily soluble in the Coupling Buffer. Water-miscible solvents are compatible with the coupling reaction at final concentrations up to 20%. The following solutions have been validated for use with this kit: 20% DMSO, DMF or ethanol; 1% Tween™-20; and 4 M urea.
- Particulate material in samples can impede column flow. Remove particulate by centrifugation (10,000 × *g*) or filtration (0.45 µm) before applying to the column.

Procedure for Peptide Immobilization

Note: Perform all centrifugations at 1,000 × *g* for 1 minute using a 15 ml collection tube.

A. Prepare the Sample for Coupling

1. Dissolve or dilute 0.1-1 mg peptide in 2 ml of Coupling Buffer. If peptide is oxidized, perform the TCEP reduction.
2. Add 0.1 ml TCEP (25 mM TCEP) to the 2 ml peptide in Coupling Buffer.
3. Incubate mixture at room temperature for 30 minutes. Equilibrate the SulfoLink Column during this incubation step.

B. Couple the Peptide to the SulfoLink Column

1. Suspend the SulfoLink Resin by mixing. Remove the top cap and then bottom closure to avoid drawing air into the resin bed.
2. Centrifuge to remove the storage buffer.
3. Add 2 ml of Coupling Buffer and centrifuge. Repeat this step two times. Replace the bottom cap.
4. Add 2-3 ml of the sulfhydryl-containing peptide.

   Note: Save 0.1 ml peptide to determine coupling efficiency.
5. Reseal the column by inverting the original snap-off closure, and with a slight twisting motion, press it firmly to the bottom tip of the column, then seal the top cap and mix by rocking or end-over-end mixing at room temperature for 15 minutes.
6. Place the column upright and incubate at room temperature for 30 minutes without mixing.
7. Remove top cap and bottom closure, place column into a new tube, and centrifuge to collect non-bound peptide.
8. Save the flow-through and determine the coupling efficiency while continuing the blocking step. Determine the coupling efficiency by comparing the peptide concentrations of the non-bound fraction to the starting sample and standards.
9. Wash the column with at least 2 ml of Wash Solution and centrifuge. Repeat this wash three times.
10. Wash the column with 2 ml of Coupling Buffer and centrifuge. Repeat this step once.

C. Block Nonspecific Binding Sites

1. Reseal the column by inverting the original snap-off closure, and with a slight twisting motion, press it firmly to the bottom tip of the column.
2. Add 15.8 mg L-Cysteine•HCl to 2 ml of Coupling Buffer (50 mM cysteine). Apply the cysteine solution to the column and replace the top cap.
3. Mix for 15 minutes at room temperature. Incubate the reaction without mixing for 30 minutes.

4. Sequentially remove the top and bottom caps and allow column to drain.
5. Either prepare the column for storage or proceed to the General Protocol for Affinity Purification Section.

D. Prepare Column for Storage

1. To equilibrate the column for storage, add 2 ml of degassed buffer (e.g., phosphate-buffered saline with optional 0.05% sodium azide) and centrifuge. Repeat this step three times.
2. Replace the bottom closure and add 2 ml of degassed buffer. Replace the top cap and store the column upright at 4°C or remove the bottom closure and proceed with the General Protocol for Affinity Purification.

General Protocol for Affinity Purification

The following protocol details a spin-purification method; if desired, the traditional gravity-flow method can be used instead. 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.

Note: For gravity-flow methods, a porous disc placed just above the resin bed automatically stops column flow when the solution has drained down to the top of the resin bed, preventing the column from drying. The disc also prevents resuspension of the packed bed when adding solution to the column. To insert the disc, use the open tube end of a resin separator to slide it to within 1 mm of the resin bed.

Materials Required

- Binding/Wash Buffer: Use phosphate-buffered saline (PBS; Product No. 28372), Tris-buffered saline (TBS; Product No. 28379) or other buffer that is conducive to forming the intended affinity interaction. Degas buffers to avoid introducing bubbles into the resin bed that may impede flow.
- Sample: Dissolve or exchange sample into Binding/Wash Buffer
- Elution Buffer: Pierce IgG Elution Buffer (Product No. 21004) or 0.1-0.2 M glycine•HCl at pH 2.5-3.0
- Neutralization Buffer (optional): Prepare 1 ml of 1 M sodium phosphate or 1 M Tris•HCl at pH 8.5-9.0

Method

Note: Perform all centrifugations at 1,000 × *g* for 1 minute using a 15 ml collection tube.

1. Equilibrate the prepared affinity column to room temperature.
2. Remove the top cap and bottom column closure. Centrifuge column to remove storage solution. Equilibrate column with 6 ml of Binding/Wash Buffer.
3. Add sample (≤ 2 ml) in appropriate binding buffer to the column. Allow sample to enter the resin bed and reseal the column by inverting the original snap-off closure, and with a slight twisting motion, press it firmly to the bottom tip of the column. Add 0.2 ml of Binding/Wash Buffer. Replace top cap and incubate column at room temperature while rocking to allow binding to occur (e.g., 15-60 minutes). For samples > 2 ml, add volumes in succession or process sample by batch method.
4. Remove the top cap and bottom closure and centrifuge column. Without changing collection tubes, add 1 ml of Binding/Wash Buffer and centrifuge again. Save the entire flow-through to evaluate binding efficiency and capacity.
5. To wash the resin, add 2 ml of Binding/Wash Buffer and centrifuge. Repeat this step 2-4 times.
6. Elute the protein with 2 ml of Elution Buffer collecting into a centrifuge tube containing 100 µl of Neutralization Buffer and centrifuge. Save eluted, neutralized sample and repeat this step 2-3 times.
7. Use the protein directly for SDS-PAGE or analyze by protein assay. If required for the specific downstream assay or storage, perform a buffer exchange by dialysis or gel filtration.

   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 ~10 times, depending on the stability of the immobilized molecule.
8. To equilibrate column, apply 4 ml of Binding/Wash Buffer and allow it to flow through the column.
9. Cap the bottom and add 4 ml of Binding/Wash Buffer that contains a final concentration of 0.05% sodium azide for long-term storage. Cap the top and store column upright at 4°C. Do not freeze the resin.

Troubleshooting

Problem	Cause	Solution
Peptide precipitates in Coupling Buffer	Peptide was not soluble in Coupling Buffer.	Dissolve peptide in a small amount of solvent and then add Coupling Buffer – see the Important Product Information and Additional Information Sections.
Low coupling efficiency	Disulfide bonds formed with time.	Reduce peptide with 1-50 mM TCEP.
Affinity column has reduced binding capacity after several uses	Immobilized peptide was damaged by time, temperature or elution conditions.	Prepare a new affinity column.
	Binding sites and resin pores had become blocked with particulate or nonspecifically bound material.	Remove precipitate from sample before affinity purification by centrifugation or 0.45 µm filter.
		Use nonionic detergent, high salt concentration, or other additives to reduce nonspecific binding or wash with greater stringency before elution.

Related Products

77720 **Bond-Breaker TCEP Solution, Neutral pH,** 5 ml
20401 **SulfoLink Coupling Resin,** 10 ml
28372 **BupH Phosphate Buffered Saline Pack,** 40 packs
22582 **Ellman's Reagent,** 5 g
44995 **SulfoLink Immobilization Kit for Proteins**

General References

Domen, P.L., *et al.* (1990). Site-directed immobilization of proteins. *J. Chromatogr*. **510:** 293-302.

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:** 91-94.

Wong, S.S. (1991). Chemistry of Protein Conjugation and Crosslinking. CRC Press: Boca Raton, FL, p. 248-251.

Product References

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

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For descriptions of symbols on product labels or product documents, go to thermofisher.com/symbols-definition. The information in this guide is subject to change without notice.

Revision history: Pub. No. MAN0011606 B

Revision	Date	Description
B	31 July 2024	Correcting spin column usage.
A	17 October 2015	New document for SulfoLink™ Immobilization Kit for Peptides.