

# SulfoLink™ Immobilization Trial Kit

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**20325**

Number	Description
20325	<p><b>SulfoLink Immobilization Trial Kit</b>, sufficient reagents to prepare one reusable affinity column</p> <p><b>Kit Contents:</b></p> <p><b>SulfoLink Column</b>, 1 × 2 ml, 6% crosslinked beaded agarose supplied as a 50% slurry in storage buffer (10 mM EDTA-Na, 0.05% NaN<sub>3</sub>, 50% glycerol)</p> <p>Binding Capacity: ≥ 10 mg reduced human IgG or ~2 mg sulfhydryl-containing peptide</p> <p><b>SulfoLink Sample Preparation Buffer</b>, 7.5 ml, 0.1 M sodium phosphate, 5 mM EDTA-Na; pH 6.0</p> <p><b>SulfoLink Coupling Buffer</b>, 120 ml, 50 mM Tris, 5 mM EDTA-Na; pH 8.5</p> <p><b>Wash Solution</b>, 25 ml, 1.0 M NaCl, 0.05% NaN<sub>3</sub></p> <p><b>2-Mercaptoethylamine•HCl</b> (2-MEA, MW = 113.61), 6 mg</p> <p><b>Bond-Breaker® TCEP Solution, Neutral pH</b>, 0.5 ml, contains stabilized aqueous 0.5 M TCEP</p> <p><b>L-Cysteine•HCl</b>, 100 mg</p> <p><b>Zeba™ Desalt Spin Columns</b>, 5 ml, 5,000 MW exclusion limit</p> <p><b>BupH™ Phosphate Buffered Saline Pack</b>, 1 pack, yields 0.1 M phosphate, 0.15 M sodium chloride; pH 7.2, when reconstituted with 500 ml of water</p> <p><b>Column Accessories</b>, porous discs (2), resin separator, and column extender</p>

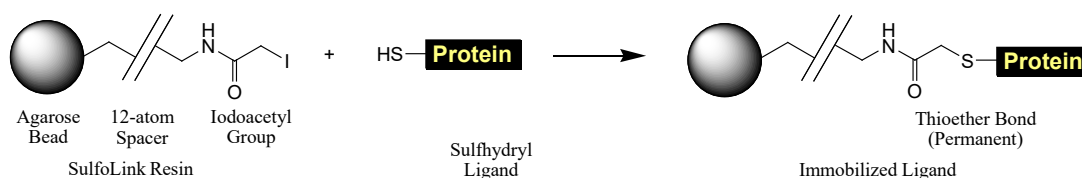
**Storage:** Upon receipt store product in the dark at 4°C. Product is shipped at ambient temperature.

## Introduction

The SulfoLink Immobilization Trial Kit contains all the necessary components for covalent attachment of sulfhydryl-containing proteins and peptides 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.

Antibodies and peptides for immobilization must have free (reduced) sulfhydryls. Two reducing agents are included in this trial kit. 2-Mercaptoethylamine•HCl (2-MEA) is a mild reducing agent suitable for selectively cleaving hinge-region disulfide bonds between IgG heavy chains while preserving the disulfide bonds between the heavy and light chains. 2-MEA must be removed by desalting before the immobilization reaction. Tris(2-carboxyethyl)phosphine (TCEP) efficiently reduces peptides and other disulfide-containing molecules but does not interfere with iodoacetyl coupling. TCEP is ideal for peptides and SulfoLink Chemistry because no removal of excess reagent is required before the immobilization reaction; however, TCEP may reduce all disulfide bonds in proteins and, therefore, is not the preferred reductant for antibodies.

Once the desired molecule is immobilized on the SulfoLink Resin, the resulting affinity column can be used for studying a variety of protein-protein interactions. The 12-atom spacer arm minimizes steric hindrance, ensuring efficient binding interactions. Depending on the stability of the immobilized molecule, 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 SulfoLink Immobilization Kit 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 protein or 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 protein, use Ellman's Reagent (Product No. 22582) according to the product instructions.
- Hydrophobic molecules may require additional wash steps or 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 at [www.embl-heidelberg.de/cgi/pi-wrapper.pl](http://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 in 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 the sample to the column.

## Procedure for Protein or Peptide Immobilization

**Note:** Perform all Zeba and SulfoLink Column centrifugations at 1,000 × g for 1 minute using a 15 ml collection tube. Do not allow the SulfoLink Resin to become dry at any time.

### A. Prepare the Protein or Peptide for Coupling

#### • Protein

1. Dissolve or dilute 1-10 mg protein with 1 ml of Sample Preparation Buffer. To ensure efficient desalting, use ≤ 1 ml of sample.
2. Add the protein solution to the vial containing 6 mg of 2-MEA (50 mM). Incubate mixture at 37°C for 1.5 hours.
3. Remove the top cap and then the bottom tab from the Desalt Spin Column and allow it to drain.
4. To equilibrate the desalt column, add 2 ml of Coupling Buffer and centrifuge. Repeat this step once.
5. Cool the reduced protein sample and slowly apply it to the center of the compact resin bed. After the sample has entered the resin, add 0.1 ml of Coupling Buffer and centrifuge. The collected solution contains the reduced protein. If desired, quantify recovery by protein assay.
6. Add 1-2 ml of Coupling Buffer to the reduced sample and continue with coupling. Save 0.1 ml of the reduced sample for later determination of coupling efficiency.

#### • Peptide

1. Dissolve or dilute 0.1-1 mg peptide in 2-3 ml of Coupling Buffer. If peptide is oxidized, perform the TCEP reduction.
2. Add 0.1-0.15 ml TCEP (25 mM TCEP) to the 2-3 ml peptide in Coupling Buffer.
3. Incubate mixture at room temperature for 30 minutes. Equilibrate the SulfoLink Column (Steps B1-B2) during this incubation step.

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**B. Couple Protein or Peptide to the SulfoLink Column**

1. Suspend the SulfoLink Resin by end-over-end mixing. To avoid drawing air into the column, sequentially remove the top cap and then the bottom closure (SAVE closure for later use). Centrifuge the column to remove the storage buffer.
2. Add 2 ml of Coupling Buffer and centrifuge. Repeat with an additional 2 ml Coupling Buffer. Replace the bottom cap.
3. Add 2-3 ml of the sulfhydryl-containing molecule to the SulfoLink Column.
4. Replace the bottom closure by inverting the original snap-off closure from the column bottom, and with a slight twisting motion, press it firmly to the bottom tip of the column. Replace the top cap and mix by rocking or end-over-end mixing at room temperature for 15 minutes.
5. Place the column upright and incubate at room temperature for 30 minutes without mixing.
6. Sequentially remove top and bottom column caps, place column into a new tube and centrifuge to collect non-bound protein or peptide.
7. Save the flow-through and determine the coupling efficiency while continuing the blocking steps. Determine the coupling efficiency by comparing the protein or peptide concentrations of the non-bound fraction to the starting sample and standards.
8. Wash the column with at least 2 ml of Wash Solution and centrifuge. Repeat this wash three times.
9. 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. 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 2 ml of degassed buffer, replace the top cap, and store the column upright at 4°C.

**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  $1,000 \times g$  for 1 minute using a 15 ml collection tube.

1. Equilibrate the prepared affinity column to room temperature.
2. Remove the top caps and bottom closure (SAVE bottom closure for later use). Centrifuge column to remove storage solution. Equilibrate column with 6 ml of Binding/Wash Buffer.
3. Add sample ( $\leq 2$  ml) in appropriate binding buffer to the column. Allow sample to enter the resin bed and replace bottom cap. 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 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 flowthrough 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  $\mu$ l 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  $\sim 10$  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. 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 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 the 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 were not reduced or sulfhydryls reformed disulfide bonds.	After reducing proteins with 2-MEA, proceed immediately with desalting and coupling procedure to prevent reformation of disulfide bonds.
		Reduce peptide with 1-50 mM TCEP.
	2-MEA was not removed from sample.	Process reduced protein through the desalt column.
Affinity column has reduced binding capacity after several uses	Immobilized ligand was damaged by time, temperature or elution conditions.	Prepare a new affinity column.
	Binding sites and resin pores became blocked with particulate or nonspecifically bound material.	Remove precipitate from sample before affinity purification by centrifugation or 0.45 $\mu$ m filter.
		Use nonionic detergent, high salt concentration, or other additives to reduce nonspecific binding or wash with greater stringency before elution.

## Related Thermo Scientific Products

89891	Zeba Desalt Spin Columns, 5 ml, 5 columns, for 500-2,000 µl samples
89892	Zeba Desalt Spin Columns, 5 ml, 25 columns, for 500-2,000 µl samples
20401	SulfoLink Coupling Resin, 10 ml
20408	2-Mercaptoethylamine•HCl (2-MEA), 6 × 6 mg in amber screw-cap vials
77720	Bond-Breaker TCEP Solution, Neutral pH, 5 ml
28372	BupH Phosphate Buffered Saline Pack, 40 packs
22582	Ellman's Reagent, 5 g
44999	SulfoLink Immobilization Kit for Peptides
44995	SulfoLink Immobilization Kit for Proteins

## General References

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Thermo Fisher Scientific | 3747 N. Meridian Road | Rockford, Illinois 61101 USA

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

Revision history: Pub. No. MAN0011608 B

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

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