

# SulfoLink<sup>®</sup> Immobilization Kit for Proteins

44995

1981.1

Number	Description
44995	<p><b>SulfoLink Immobilization Kit for Proteins</b>, sufficient reagents to prepare five reusable affinity columns</p> <p><b>Kit Contents:</b></p> <p><b>SulfoLink Column</b>, 5 × 2mL, 6% crosslinked beaded agarose supplied as a 50% slurry in storage buffer (10mM EDTA-Na, 0.05% NaN<sub>3</sub>, 50% glycerol)            Binding Capacity: ≥ 5mg reduced human IgG/mL of resin</p> <p><b>SulfoLink Sample Preparation Buffer</b>, 7.5mL, 0.1M sodium phosphate, 5mM EDTA-Na; pH 6.0</p> <p><b>SulfoLink Coupling Buffer</b>, 500mL, 50mM Tris, 5mM EDTA-Na; pH 8.5</p> <p><b>Wash Solution</b>, 120mL, 1.0 M NaCl, 0.05% NaN<sub>3</sub></p> <p><b>2-Mercaptoethylamine•HCl</b> (2-MEA, MW = 113.61), 5 × 6mg</p> <p><b>L-Cysteine•HCl</b>, 100mg</p> <p><b>Zeba™ Desalt Spin Columns</b>, 5 × 5mL, 5,000 MW exclusion limit</p> <p><b>BupH™ Phosphate Buffered Saline Pack</b>, 1 pack, yields 0.1M phosphate, 0.15M sodium chloride; pH 7.2, when reconstituted with 500mL of water</p> <p><b>Column Accessories</b>, porous discs (6), white tips (5), resin separator and column extender</p>

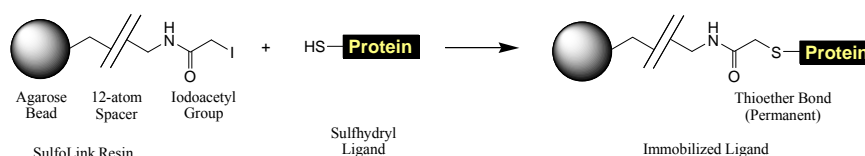
**Storage:** Upon receipt store product in the dark at 4°C. Product is shipped at ambient temperature.

## Introduction

The Thermo Scientific SulfoLink Immobilization Kit for Proteins contains all the necessary components for covalent attachment of sulfhydryl-containing proteins and other ligands to a beaded agarose support. The SulfoLink 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 other molecules for immobilization must have free (reduced) sulfhydryls. 2-Mercaptoethylamine•HCl (2-MEA), included in the kit, 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. The result is two functional half antibodies that have sulfhydryls available for immobilization. In contrast, other disulfide-reducing agents will cleave all disulfide bonds in IgG molecules at nearly equal rates, destroying antigen-binding function. Therefore, 2-MEA is optimal for selective, partial reduction of antibodies.

Once the desired molecule is immobilized, 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 for Proteins 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 and estimate coupling efficiency use a spectrophotometer or other suitable method.
- To determine if free sulfhydryls are available on the protein, use Ellman's Reagent (Product No. 22582) according to the product instructions.
- Hydrophobic proteins may require additional wash steps or detergent during coupling to minimize nonspecific binding.
- Particulate material in samples can impede column flow. Remove particulate by centrifugation ( $10,000 \times g$ ) or filtration ( $0.45 \mu\text{m}$ ) before applying the sample to the column.

## Procedure for Protein Immobilization

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

### A. Prepare the Protein for Coupling

1. Dissolve or dilute 1-10mg protein with 1mL of Sample Preparation Buffer. To ensure efficient desalting, use  $\leq 1\text{mL}$  of sample.
2. Add the protein solution to the vial containing 6 mg of 2-MEA (50mM). Incubate mixture at  $37^\circ\text{C}$  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 2mL 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 0.1mL of Coupling Buffer and centrifuge. The collected solution contains the reduced protein. If desired, quantify recovery by protein assay.
6. Add 1-2mL of Coupling Buffer to the reduced sample and continue with coupling. Save 0.1mL of the reduced sample for later determination of coupling efficiency.

### B. Couple Protein 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 tab. Centrifuge the column to remove the storage buffer.
2. Add 2mL of Coupling Buffer and centrifuge. Repeat with an additional 2mL Coupling Buffer. Replace the bottom cap.
3. Add 2-3mL of the sulfhydryl-containing protein to the SulfoLink Column.
4. Replace the bottom and 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.
7. Save the flow-through and determine the coupling efficiency while continuing the blocking steps. Determine the coupling efficiency by comparing the protein concentrations of the non-bound fraction to the starting sample and standards.
8. Wash the column with at least 2mL of Wash Solution and centrifuge. Repeat this wash three times.
9. Wash the column with 2mL of Coupling Buffer and centrifuge. Repeat this step once.

### C. Block Nonspecific Binding Sites

1. Replace the bottom cap.
2. Add 15.8 mg L-Cysteine•HCl to 2mL 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 2mL 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 cap and add 2mL 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 1mm 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: Thermo Scientific IgG Elution Buffer (Product No. 21004) or 0.1-0.2M glycine•HCl at pH 2.5-3.0
- Neutralization Buffer (optional): Prepare 1mL of 1M sodium phosphate or 1M Tris•HCl at pH 8.5-9.0

#### Method

**Note:** Perform all centrifugations at  $1,000 \times g$  for 1 minute using a 15mL collection tube.

1. Equilibrate the prepared affinity column to room temperature.
2. Remove top and bottom column caps. Centrifuge column to remove storage solution. Equilibrate column with 6mL of Binding/Wash Buffer.
3. Add sample ( $\leq 2\text{mL}$ ) in appropriate binding buffer to the column. Allow sample to enter the resin bed and replace bottom cap. Add 0.2mL 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  $> 2\text{mL}$ , add volumes in succession or process sample by batch method.
4. Remove top and bottom caps and centrifuge column. Without changing collection tubes, add 1mL of Binding/Wash Buffer and centrifuge again. Save the entire flow-through to evaluate binding efficiency and capacity.
5. To wash the resin, add 2mL of Binding/Wash Buffer and centrifuge. Repeat this step 2-4 times.
6. Elute the protein with 2mL of Elution Buffer collecting into a centrifuge tube containing 100  $\mu\text{L}$  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 4mL of Binding/Wash Buffer and allow it to flow through the column.
9. Cap the bottom and add 4mL 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
Low coupling efficiency	Disulfide bonds were not reduced or reformed	Proceed immediately with desalting and coupling procedure after 2-MEA reaction to prevent reformation of disulfide bonds
	2-MEA not removed from sample	Process reduced protein through the desalt column
Affinity column has reduced binding capacity after several uses	Immobilized protein was damaged by time, temperature or elution conditions	Prepare a new affinity column
	Binding sites and resin pores have 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

## Additional Information

Visit our website for additional information including the following items:

- Tech Tip #12: Prepare molecules with poor solubility for immobilization on affinity supports
- Tech Tip #27: Optimize elution conditions for immunoaffinity purification
- Tech Tip #29: Degas buffers for use in affinity and gel filtration columns

## Related Thermo Scientific Products

<b>89891</b>	<b>Zeba Spin Desalting Columns, 5mL</b> , 5 columns, for 500-2,000µL samples
<b>89892</b>	<b>Zeba Spin Desalting Columns, 5mL</b> , 25 columns, for 500-2,000µL samples
<b>20408</b>	<b>2-Mercaptoethylamine•HCl (2-MEA)</b> , 6 × 6 mg in amber screw-cap vials
<b>28372</b>	<b>BupH Phosphate Buffered Saline Pack</b> , 40 packs
<b>22582</b>	<b>Ellman's Reagent</b> , 5g

## 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.
- 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<sup>-/-</sup> 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.

This product ("Product") is warranted to operate or perform substantially in conformance with published Product specifications in effect at the time of sale, as set forth in the Product documentation, specifications and/or accompanying package inserts ("Documentation") and to be free from defects in material and workmanship. Unless otherwise expressly authorized in writing, Products are supplied for research use only. No claim of suitability for use in applications regulated by FDA is made. The warranty provided herein is valid only when used by properly trained individuals. Unless otherwise stated in the Documentation, this warranty is limited to one year from date of shipment when the Product is subjected to normal, proper and intended usage. This warranty does not extend to anyone other than the original purchaser of the Product ("Buyer").

**No other warranties, express or implied, are granted, including without limitation, implied warranties of merchantability, fitness for any particular purpose, or non infringement. Buyer's exclusive remedy for non-conforming Products during the warranty period is limited to replacement of or refund for the non-conforming Product(s).**

There is no obligation to replace Products as the result of (i) accident, disaster or event of force majeure, (ii) misuse, fault or negligence of or by Buyer, (iii) use of the Products in a manner for which they were not designed, or (iv) improper storage and handling of the Products.

Current versions of product instructions are available at [www.thermo.com/pierce](http://www.thermo.com/pierce). For a faxed copy, call 800-874-3723 or contact your local distributor.

© 2010 Thermo Fisher Scientific Inc. All rights reserved. Unless otherwise indicated, all trademarks are property of Thermo Fisher Scientific Inc. and its subsidiaries. Printed in the USA.