INSTRUCTIONS

SulfoLink™ Immobilization Kit for Proteins

Pub. No. MAN0011607
Rev. B
Doc. Part No. 2161981

44995

Number	Description
44995	**SulfoLink Immobilization Kit for Proteins,** sufficient reagents to prepare five reusable affinity columns

Kit Contents:

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

Binding Capacity: ≥ 5mg reduced human IgG/mL of resin

SulfoLink Sample Preparation Buffer, 7.5mL, 0.1M sodium phosphate, 5mM EDTA-Na; pH 6.0

SulfoLink Coupling Buffer, 500mL, 50mM Tris, 5mM EDTA-Na; pH 8.5

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

2-Mercaptoethylamine•HCl (2-MEA, MW = 113.61), 5 × 6mg

L-Cysteine•HCl, 100mg

Zeba™ Desalt Spin Columns, 5 × 5mL, 5,000 MW exclusion limit

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

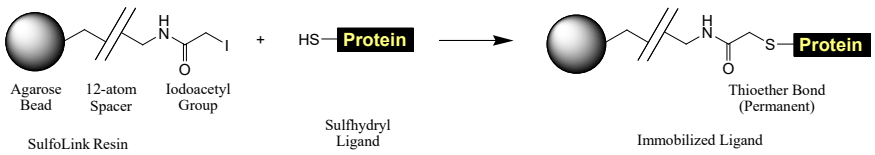

Figure 1. General structure and reaction scheme for the SulfoLink Coupling Resin.

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 × *g*) or filtration (0.45 µm) before applying the sample to the column.

Procedure for Protein Immobilization

Note: Perform all Zeba and SulfoLink Column centrifugations at 1,000 × *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 ≤ 1mL of sample.
2. Add the protein solution to the vial containing 6 mg of 2-MEA (50mM). Incubate mixture at 37°C for 1.5 hours.
3. Remove the top cap and then the bottom snap-off closure (SAVE cap for later use) 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 closure. 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 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..
3. Add 2-3mL of the sulfhydryl-containing protein to the SulfoLink Column.
4. Replace the bottom closure 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 the top cap and bottom closure, 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. 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 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 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. Add 2mL of degassed buffer and 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 × *g* for 1 minute using a 15mL collection tube.

1. Equilibrate the prepared affinity column to room temperature.
2. Remove the top cap and bottom closure. Centrifuge the column to remove storage solution. Equilibrate the column with 6mL of Binding/Wash Buffer.
3. Add sample (≤ 2mL) 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 > 2mL, 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 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 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 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 was 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 became 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 Thermo Scientific Products

89891 **Zeba Spin Desalting Columns, 5mL,** 5 columns, for 500-2,000µL samples

89892 **Zeba Spin Desalting Columns, 5mL,** 25 columns, for 500-2,000µL samples

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

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

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

General References

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Product References

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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.

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

Revision history: Pub. No. MAN0011607 B

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