

# EZ-Link™ Maleimide Protein Labeling Kit

Catalog Number C20045

Pub. No. MAN0025994 Rev. A.0

**WARNING!** Read the Safety Data Sheets (SDSs) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves. Safety Data Sheets (SDSs) are available from [thermofisher.com/support](http://thermofisher.com/support).

## Product description

The Thermo Scientific™ EZ-Link™ Maleimide Protein Labeling Kit (Cat. No. C20045) provides a convenient method to label proteins with maleimide groups for crosslinking to thiol- or sulfhydryl-containing moieties. The kit uses Sulfo-SMCC, a heterobifunctional crosslinker that readily reacts with primary amines, forming covalently attached maleimide groups that can be further reacted with thiol or cysteine residues to produce diverse bioconjugates. The kit includes reagents for maleimide-labeling and purification of five 100- $\mu$ L samples with a concentration of 1–10 mg/mL.

The kit includes the following reagents.

- **Sulfo-SMCC, No-Weigh™ Format**—Crosslinker that contains sulfonated *N*-hydroxysuccinimide (NHS) ester and maleimide reactive groups. NHS esters target primary amines at pH 7.0–9.0 to form amide bonds, while maleimides react with sulfhydryl groups at pH 6.5–7.5 to form stable thioether bonds.
- **Zeba™ Dye and Biotin Removal Spin Columns**—Contain a ready-to-use resin that is designed for rapid removal of unconjugated crosslinker with exceptional protein recovery (typically >85%).
- **BupH™ Phosphate Buffered Saline Pack**—Contains a dry-blend powder that is sufficient to prepare 500 mL of phosphate-buffered saline (PBS; 0.1 M sodium phosphate, 0.15 M NaCl, pH 7.2).

## Procedure overview

In this procedure, a 10- to 50-fold molar excess of Sulfo-SMCC is added to the protein sample. The reaction mixture is incubated at room temperature for 30 minutes, during which the sulfonated NHS ester reacts with primary amines to produce maleimide-labeled protein. After excess non-reacted Sulfo-SMCC and other byproducts are removed, the maleimide-labeled protein is incubated with a thiol coupling partner (containing a sulfhydryl group; supplied separately) to produce the final conjugated protein (see Figure 1).

**Note:** The cyclohexane ring in the spacer arm of Sulfo-SMCC decreases the rate of hydrolysis of the maleimide group compared to similar reagents that do not contain this ring. This feature enables proteins that have been maleimide-labeled with Sulfo-SMCC to be lyophilized and stored for later use in conjugation reactions.

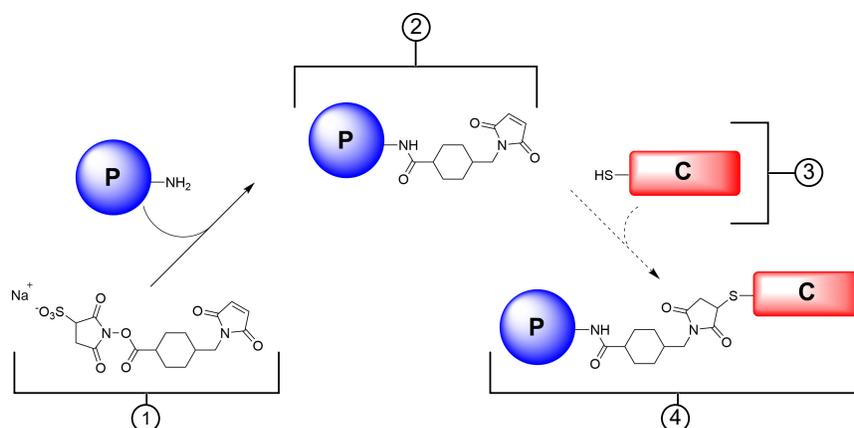


Figure 1 Two-step reaction scheme: Conjugation of a protein (P) to a thiol-containing coupling partner (C) using Sulfo-SMCC

- ① Sulfo-SMCC  
 ② Maleimide-labeled protein  
 ③ Thiol coupling partner  
 ④ Conjugated protein

## Contents and storage

**Table 1** EZ-Link™ Maleimide Protein Labeling Kit (Cat. No. [C20045](#))

Contents	Amount	Storage <sup>[1]</sup>
Sulfo-SMCC, No-Weigh™ Format	5 × 2 mg	-20°C Store desiccated.
Zeba™ Dye and Biotin Removal Spin Columns <sup>[2]</sup>	5 columns	2–8°C
BupH™ Phosphate Buffered Saline Pack	1 pack	20–25°C

<sup>[1]</sup> The kit is stable for 1 year when stored as directed.

<sup>[2]</sup> The resin is supplied in a 0.1M NaCl/0.05% sodium azide solution.

## Required materials not supplied

Unless otherwise indicated, all materials are available through [thermofisher.com](#). "MLS" indicates that the material is available from [fisherscientific.com](#) or another major laboratory supplier.

Catalog numbers that appear as links open the web pages for those products.

**Table 2** Materials required for maleimide-labeling and purification

Item	Source
Variable-speed benchtop microcentrifuge	<a href="#">MLS</a>
1.5- or 2-mL microcentrifuge tubes	<a href="#">MLS</a>
Vortex mixer	<a href="#">MLS</a>
Deionized water	<a href="#">MLS</a>

**Table 3** (Optional) Additional materials required for conjugation to a thiol-containing coupling partner

Item	Source
Zeba™ Spin Desalting Columns	<a href="#">www.thermofisher.com</a>
Slide-A-Lyzer™ Dialysis Cassettes	<a href="#">www.thermofisher.com</a>

## Procedural guidelines

- Do not use buffers that contain primary amines (e.g., Tris or glycine) or sulfhydryls because they will compete with the intended reaction. If necessary, dialyze or desalt samples into an appropriate buffer, such as PBS, before use.
- Sulfo-SMCC is soluble up to 10 mM in water and other aqueous buffers. Solubility, however, decreases with increasing salt concentration. Sulfo-SMCC can also be prepared in an organic solvent, such as anhydrous dimethylsulfoxide (DMSO) or dimethylformamide (DMF); subsequent dilution into an aqueous reaction buffer is generally possible if the final concentration of organic solvent is less than 10%.
- Sulfo-SMCC is moisture-sensitive. Equilibrate the vial to room temperature before opening to prevent moisture condensation inside the vial. Store unused vials in the foil pouch provided.
- Use Sulfo-SMCC immediately after reconstitution. Discard any unused portion.
- Ensure molecules have a free (reduced) sulfhydryl to react with the maleimide moiety of Sulfo-SMCC.
  - **To add sulfhydryls to molecules:** Use Pierce™ SATA (N-succinimidyl S-acetylthioacetate) (Cat. No. [26102](#)) or Pierce™ Traut's Reagent (2-iminothiolane) (Cat. No. [26101](#)), which modify primary amines.
  - **To reduce disulfide bonds in peptides:** Use Pierce™ Immobilized TCEP Disulfide Reducing Gel (Cat. No. [77712](#)).
  - **To reduce disulfide bonds in proteins:** Use 5 mM (1:100 dilution) of Bond-Breaker™ TCEP Solution, Neutral pH (Cat. No. [77720](#)), incubate for 30 minutes at room temperature, then pass two times through a desalting column (e.g., Zeba™ Spin Desalting Columns).

**Note:** Complete reduction of disulfide bonds can inactivate proteins (e.g., antibodies). Selective reduction of hinge-region disulfide bonds in IgG can be accomplished with Pierce™ Mercaptoethylamine-HCl (2-MEA; Cat. No. [20408](#)).

- Removal of excess Sulfo-SMCC is essential for efficient conjugation of a maleimide-activated protein to a thiol coupling partner.
- Do not reuse Zeba™ Dye and Biotin Removal Spin Columns.

## Before you begin

1. Prepare PBS solution—Add the contents of one BupH™ Phosphate Buffered Saline Pack to 500 mL of deionized water.
2. Prepare 100 µL of protein sample in PBS solution at the indicated concentration according to the sample type.
  - Antibody: 1 mg/mL (recommended)
  - Purified protein: 1–10 mg/mL

## Protein labeling procedure

**1** Prepare Sulfo-SMCC solution, then combine with the sample

- 1.1. Equilibrate one vial of Sulfo-SMCC, No-Weigh™ Format to room temperature.
- 1.2. Prepare Sulfo-SMCC solution (5 mg/mL)—Add 400 µL of deionized water to one vial of Sulfo-SMCC, No-Weigh™ Format. Vortex or pipet up and down until the solution is homogenous.

**IMPORTANT!** Use the Sulfo-SMCC solution immediately. Hydrolysis begins upon reconstitution.

- 1.3. Add the appropriate molar excess of Sulfo-SMCC solution to the prepared sample according to the following table.

Sample type	Sample concentration	Recommended molar excess	Volume of Sulfo-SMCC solution per sample
IgG antibody (100 µg)	1 mg/mL	20X	4 µL
Purified protein	1–4 mg/mL	20X	Variable
	5–10 mg/mL	5–10X	

- 1.4. Incubate the reaction mixture for 30 minutes at room temperature or 2 hours at 4°C.

Discard the unused portion of the Sulfo-SMCC solution.

**2** Prepare the Zeba™ Dye and Biotin Removal Spin Column

- 2.1. Twist to remove the bottom plug of the column, then loosen the cap. Do not remove the cap.

- 2.2. Place the column in a 1.5- or 2-mL microcentrifuge tube, then centrifuge the column-tube assembly at 1,000 × g for 2 minutes. Discard the flow-through.

If you are using a fixed angle rotor, place a mark on the side of the column facing away from the rotor. For all subsequent centrifugation steps, ensure that the column is oriented in the same position.

**IMPORTANT!** Improper orientation of the column during centrifugation can result in inefficient removal of Sulfo-SMCC.

- 2.3. (Optional) Equilibrate the column.
  - a. Remove the cap of the column, then add 300–400 µL of equilibration buffer.
  - b. Centrifuge at 1,000 × g for 2 minutes. Discard the flow-through.

**3** Purify the maleimide-labeled protein

- 3.1. Place the prepared column in a new microcentrifuge tube, then remove the cap.
- 3.2. Slowly apply the reaction mixture to the center of the settled resin.
- 3.3. Centrifuge the column-tube assembly at 1,000 × g for 2 minutes. Discard the column. The purified maleimide-labeled protein is in the microcentrifuge tube.

- 3.4. (Optional) Wash the column with 100 µL of buffer (e.g., PBS solution) to maximize sample recovery.

**Note:** Do not perform this step if a higher concentration of protein is desired.

**3** Purify the maleimide-labeled protein (*continued*) Use the maleimide-labeled protein immediately for conjugation, or store frozen or lyophilized for future use.

**4** (*Optional*) Conjugate the maleimide-labeled protein to a thiol-containing coupling partner

**4.1.** Combine the maleimide-labeled protein with the thiol-containing coupling partner (supplied separately) at an appropriate molar ratio, depending on the desired degree of conjugation.

**Note:** Optimal molar ratios for reactions must be determined empirically.

**4.2.** Incubate the reaction mixture for 30 minutes at room temperature or 2 hours at 4°C.

**Note:**

- Incubation at 4°C can be extended to several hours or overnight if needed.
- The conjugation reaction can be stopped before completion by adding buffer that contains reduced cysteine at a concentration several times greater than the concentration of sulfhydryl groups of the coupling partner.

**4.3.** Depending on your application, proceed in one of the following ways.

- Use the conjugated protein immediately for the intended application.
- Proceed to purify the conjugated protein. See “Purify the maleimide-labeled protein” on page 3.

Efficiency of the conjugation reaction can be estimated using electrophoresis separation and subsequent protein staining.

## Related products

Unless otherwise indicated, all materials are available through [thermofisher.com](http://thermofisher.com).

Product	Cat. No.
Pierce™ SATA (N-succinimidyl S-acetylthioacetate)	<a href="#">26102</a>
Pierce™ Traut's Reagent (2-iminothiolane)	<a href="#">26101</a>
Pierce™ Immobilized TCEP Disulfide Reducing Gel	<a href="#">77712</a>
Bond-Breaker™ TCEP Solution, Neutral pH	<a href="#">77720</a>
Pierce™ Mercaptoethylamine-HCl	<a href="#">20408</a>
Sulfo-SMCC, No-Weigh™ Format	<a href="#">A39268</a>
Zeba™ Dye and Biotin Removal Spin Columns	<a href="#">A44296</a>

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**Revision history:** Pub. No. MAN0025994

Revision	Date	Description
A.0	6 December 2021	New manual for EZ-Link™ Maleimide Protein Labeling Kit.

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