SMCC and Sulfo-SMCC

Catalog Numbers 22122, 22322, 22360, A35394, and A39268

Doc. Part No. 2160581 Pub. No. MAN0011295 Rev. C.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**.

Product description

The Thermo Scientific™ SMCC and its water-soluble analog Sulfo-SMCC are heterobifunctional crosslinkers that contain *N*-hydroxysuccinimide (NHS) ester and maleimide groups that allow covalent conjugation of amine- and sulfhydryl-containing molecules. NHS esters react with 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. In aqueous solutions, NHS ester hydrolytic degradation is a competing reaction whose rate increases with pH. The maleimide group is more stable than the NHS-ester group but will slowly hydrolyze and lose its reaction specificity for sulfhydryls at pH values > 7.5. For these reasons, conjugations with these crosslinkers are usually performed at pH 7.2–7.5, with the NHS-ester (amine-targeted) reaction occurring before or simultaneously with the maleimide (sulfhydryl-targeted) reaction.

The cyclohexane ring in the spacer arm of these reagents 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-activated with SMCC or Sulfo-SMCC to be lyophilized and stored for later conjugation to a sulfhydryl-containing molecule. Many maleimide-activated protein products are produced in this manner (see “Related products” on page 4).

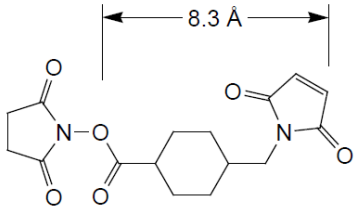
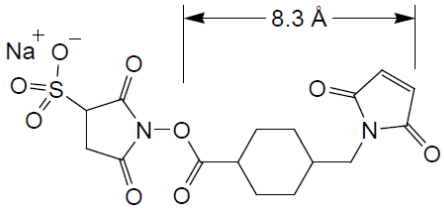
SMCC and Sulfo-SMCC are often used to prepare antibody-enzyme and hapten-carrier protein conjugates in a two-step reaction scheme. First, the amine-containing protein is reacted with a several-fold molar excess of the crosslinker, followed by removal of excess (nonreacted) reagent by desalting or dialysis. Finally, the sulfhydryl-containing molecule is added to react with the maleimide groups that are already attached to the first protein.

Sulfo-SMCC is soluble in water and many other aqueous buffers up to approximately 5 mg/mL, although solubility decreases with increasing salt concentration. SMCC is not directly water-soluble and must be dissolved in an organic solvent, such as dimethylsulfoxide (DMSO) or dimethylformamide (DMF); subsequent dilution into aqueous reaction buffer is generally possible, and most protein reactants will remain soluble if the final concentration of organic solvent is less than 10%.

Contents and storage

SMCC and Sulfo-SMCC reagents are shipped at room temperature. Upon receipt, store the reagents according to the following table:

Table 1 SMCC and Sulfo-SMCC reagents

Product[1]	Amount	Cat. No.	Properties	Storage
SMCC (succinimidyl 4-[*N*-maleimidomethyl]cyclohexane-1-carboxylate)	50 mg	22360	Molecular weight: 334.32; Spacer arm: 8.3Å; Net mass added: 219.09 	4°C Store desiccated.
SMCC, No-Weigh™ Format	10 × 1 mg microtubes	A35394		
Sulfo-SMCC (sulfosuccinimidyl 4-[*N*-maleimidomethyl]cyclohexane-1-carboxylate)	1 g	22122	Molecular weight: 436.37; Spacer arm: 8.3Å; Net mass added: 219.09; CAS #: 92921-24-9 	–20°C Store desiccated.
Sulfo-SMCC	50 mg	22322		
Sulfo-SMCC, No-Weigh™ Format	10 × 2 mg microtubes	A39268		

[1] Product labels are provided in an aluminum foil pouch for your convenience. We recommend labeling vials to avoid any confusion as you work with the reagent.

Procedural guidelines

- SMCC and Sulfo-SMCC are moisture-sensitive. Store desiccated. Equilibrate vial to room temperature before opening to avoid moisture condensation inside the container. Dissolve needed amount of reagent and use it immediately before hydrolysis occurs. Discard any unused reconstituted reagent. Do not store reagent in solution.
- No-Weigh™ microtube handling: Immediately before use, uncap and add desired solvent. Sulfo-SMCC is water soluble and can be dissolved in 400 µL of ultrapure water. Pipette up and down to mix. Alternatively, vortex for a few seconds to ensure a homogeneous solution. The maximum useable volume of the vial is 800 µL.

  Note: Do not use phosphate-buffered saline (PBS) for initial dissolution of Sulfo-SMCC; the reagent does not dissolve well in buffers exceeding 50 mM total salts. However, once dissolved, the solution can be further diluted in PBS or other non-amine buffers.
- After use, discard any remaining solution.
- Avoid buffers containing primary amines (e.g., Tris or glycine) and sulfhydryls during conjugation, because they will compete with the intended reaction. If necessary, dialyze or desalt samples into an appropriate buffer, such as phosphate-buffered saline (PBS).
- A molecule must have a free (reduced) sulfhydryl to react with the maleimide moiety of SMCC and Sulfo-SMCC reagents. To reduce peptide disulfide bonds, we recommend Thermo Scientific™ Immobilized TCEP Disulfide Reducing Gel (Cat No. 77712). For proteins, reduce disulfide bonds using 5 mM TCEP (1:100 dilution of Thermo Scientific™ Bond-Breaker™ TCEP Solution, Cat. No. 77720) for 30 minutes at room temperature, followed by two passes through a suitable desalting column (e.g., Thermo Scientific™ Zeba™ Spin Desalting Columns). Be aware that proteins (e.g., antibodies) may be inactivated by complete reduction of their disulfide bonds. Selective reduction of hinge-region disulfide bonds in IgG can be accomplished with 2-Mercaptoethylamine•HCl (2-MEA, Cat. No. 20408). Sulfhydryls can be added to molecules using N-succinimidyl S-acetylthioacetate (SATA, Cat. No. 26102) or 2-iminothiolane•HCl (Pierce™ Traut's Reagent, Cat. No. 26101), which modify primary amines.

Procedure for two-step protein crosslinking

Generally, a 10- to 50-fold molar excess of crosslinker over the amount of amine-containing protein results in sufficient maleimide activation to enable several sulfhydryl-containing proteins to be conjugated to each amine-containing protein. More dilute protein solutions require greater fold molar excess of reagent to achieve the same activation level. Empirical testing is necessary to determine optimal activation levels and final conjugation ratios for the intended application.

Before you begin

- Prepare conjugation buffer: Phosphate-buffered saline (PBS = 100 mM sodium phosphate, 150 mM sodium chloride, pH 7.2; e.g., Cat. No. 28372) or other amine- and sulfhydryl-free buffer at pH 6.5–7.5 (see "Procedural guidelines" on page 2) The addition of 1–5 mM EDTA can help to chelate divalent metals, thereby reducing disulfide formation in the sulfhydryl-containing protein.
- Prepare desalting columns to separate the modified protein from excess crosslinker and reaction byproducts (e.g., Zeba™ Spin Desalting Columns).
- Prepare amine-containing (Protein-NH_2) and sulfhydryl-containing proteins (Protein-SH) in Conjugation Buffer using standard laboratory procedures.

Protocol

Note: Before starting this procedure, ensure that Protein-SH is prepared and ready to combine with Protein-NH_2.

1. Determine the appropriate molar excess of SMCC or Sulfo-SMCC crosslinker based on the concentration of Protein-NH_2.
   - For protein samples <1 mg/mL: Use 40- to 80-fold molar excess.
   - For protein samples of 1–4 mg/mL: Use 20-fold molar excess.
   - For protein samples of 5–10 mg/mL: Use 5- to 10-fold molar excess.

   Note: If the Sulfo-SMCC solution does not completely dissolve, place the tube under hot running water or incubate for several minutes in a 50°C water bath.

2. Prepare the crosslinker, then combine with Protein-NH_2. For recommended crosslinker concentrations and volumes, see Table 2.
   a. Dissolve the crosslinker in the appropriate solvent and at the concentration denoted in parentheses.
   b. Add the indicated volume of crosslinker to 1 mL of Protein-NH_2.

      Example: For reacting a 1 mL solution of Protein-NH_2 sample at a concentration of 10 mg/mL with No-Weigh™ Sulfo-SMCC (2 mg), first dissolve the contents of the tube in 400 µL of ultra pure water, then add the prescribed volume (100 µL) to the 1 mL sample.

      Note: If you are using Sulfo-SMCC or SMCC, the appropriate amount of dry reagent must be weighed on a balance (e.g., 2.4 mg Sulfo-SMCC for dissolution in 500 µL buffer).

Table 2 Crosslinker preparation and molar excess to use for 1 mL of sample

Protein-NH_2 concentration (based on a 50kDa protein)	10 mg/mL	1 mg/mL	0.5 mg/mL
Crosslinker Molar Excess	**5X**	**20X**	**50X**
Sulfo-SMCC (in 50 mM sodium phosphate or water)	100 µL (5 mg/mL[1])	40 µL (5 mg/mL[1])	50 µL (5 mg/mL[1])
No-Weigh™ Sulfo-SMCC (in 50 mM sodium phosphate or water)	100 µL (5 mg/mL[1])	40 µL (5 mg/mL[1])	50 µL (5 mg/mL[1])
SMCC (in DMSO or DMF)	100 µL (3.7 mg/mL[1])	100 µL (1.5 mg/mL[1])	100 µL (1.8 mg/mL[1])

[1] Concentration of each crosslinker before adding to protein sample.

Note: If the Sulfo-SMCC solution does not completely dissolve, place the tube under hot running water or incubate for several minutes in a 50°C water bath.

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

4. Remove excess crosslinker using a desalting column equilibrated with Conjugation Buffer.

   Note: We recommend Zeba™ Dye and Biotin Removal Columns.

5. Combine Protein-SH and desalted Protein-NH_2 in a molar ratio corresponding to that desired for the final conjugate and consistent with the relative number of sulfhydryl and activated amines that exist on the two proteins.

6. Gently mix, then incubate the reaction mixture at room temperature for 30 minutes or 2 hours at 4°C.

   Note: Generally, there is no harm in allowing the reaction to proceed for several hours or overnight, although usually the reaction will be complete in the specified time. To stop the conjugation reaction before completion, add buffer containing reduced cysteine at a concentration several times greater than the sulfhydryls of Protein-SH.

   Note: Conjugation efficiency can be estimated by electrophoresis separation and subsequent protein staining.

Two-step reaction scheme

In this example, the crosslinker is first reacted with the antibody to produce a maleimide-activated protein. After excess non-reacted crosslinker and by-products are removed, the maleimide-activated antibody is reacted with the appropriate molar ratio of enzyme having sulfhydryl groups. Usually, several or multiple maleimide-activations occur per antibody molecule, enabling several enzyme molecules to be conjugated to each antibody molecule.

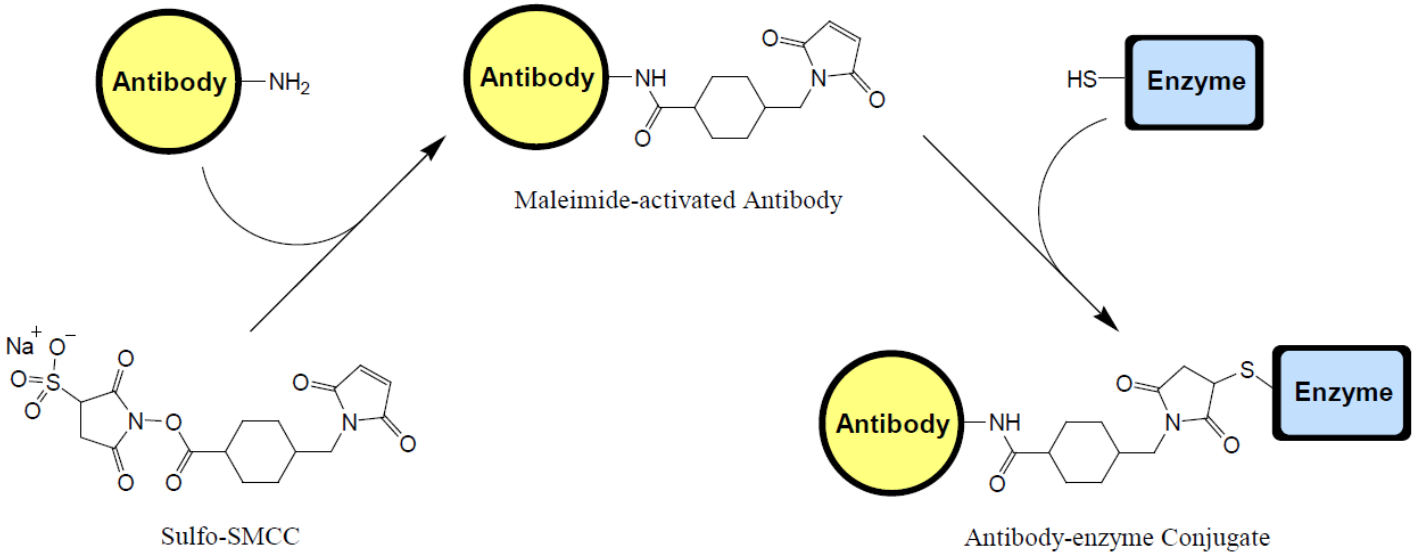


Figure 1 Two-step reaction scheme for conjugating antibody and enzyme proteins with Sulfo-SMCC.

Related products

Table 3 Non-cleavable NHS/Maleimide crosslinkers

Crosslinker name	Spacer arm length (Å)	Spacer arm composition (between ester and maleimide)	Cat. No. (NHS)	Cat. No. (Sulfo-NHS)
AMAS	4.4	Alkane	22295	NA
BMPS	5.9	Alkane	22298	NA
GMBS	7.3	Alkane	22309	22324
MBS	7.3	Aromatic	22311	22312
SMCC	8.3	Cyclohexane	22360	22322
EMCS	9.4	Alkane	22308	22307
SMPB	11.6	Alkane/Aromatic	22416	22317
SMPH	14.2	Alkane/Amide	22363	NA
LC-SMCC	16.2	Alkane/Amide/Cyclohexane	22362	NA
KMUS	16.3	Alkane	NA	21111

Table 4 Thermo Scientific™ Maleimide crosslinkers

Product	Amount	Cat. No.
Maleimide-Activated NeutrAvidin™ Protein	5 mg	31007
EZ-Link™ Maleimide-Activated Horseradish Peroxidase	5 mg	31485
Imject™ Maleimide Activated Mariculture Keyhole Limpet Hemocyanin (mcKLH)	2 mg	77606

Limited product warranty

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Revision history: Pub. No. MAN0011295

Revision	Date	Description
C.0	24 January 2022	• The solubility of Sulfo-SMCC in water and aqueous buffers was corrected from 10 mM to 5 mg/mL. • The document was updated to the current template, with associated updates to the limited license information, warranty, trademarks, and logos.

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