INSTRUCTIONS

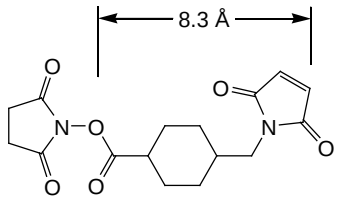
SMCC and Sulfo-SMCC

MAN0011295
Rev. B.0
Pub. Part No. 2160581

22122 22360 22322 A39268

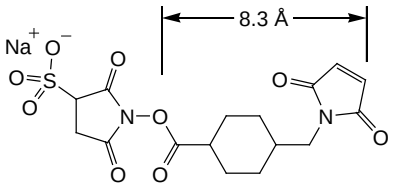
Number	Description
22360	**SMCC** (succinimidyl 4-[*N*-maleimidomethyl]cyclohexane-1-carboxylate), 50mg

Molecular Weight: 334.32
Spacer Arm: 8.3Å
Net Mass Added: 219.09
Storage: Upon receipt store desiccated at 4°C. Product is shipped at ambient temperature.



22122	**Sulfo-SMCC** (sulfosuccinimidyl 4-[*N*-maleimidomethyl]cyclohexane-1-carboxylate), 1g
22322	**Sulfo-SMCC,** 50mg
A39268	**Sulfo-SMCC, No-Weigh Format,** 10 × 2mg

Molecular Weight: 436.37
Spacer Arm: 8.3Å
Net Mass Added: 219.09
CAS #: 92921-24-9



Storage: Upon receipt store desiccated at -20°C. Product is shipped at ambient temperature.
Note: Product labels have been provided for your convenience. Please label the vials using one of the labels provided in the Al foil pouch to avoid any confusion as you work with this No-Weigh reagent.

Introduction

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 7-9 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 loses 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) reacted before or simultaneous 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.[1] 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 Thermo Scientific Products).

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 already attached to the first protein.

Sulfo-SMCC is soluble in water and many other aqueous buffers to approximately 10mM, 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%.

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Important Product Information

- SMCC and Sulfo-SMCC are moisture-sensitive. Store reagent vial in desiccant. 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.
- Thermo Scientific™ No-Weigh™ Tube Handling: Immediately before use, uncap the vial and add 200µL of 50mM sodium phosphate buffer (pH 7.0-7.5) or ultrapure water and pipette up and down to mix. Alternatively, vortex for a few seconds to ensure a homogeneous solution. After use, discard any remaining solution. Store the unused vials in the foil pouch provided. **The maximum useable volume of the vial is 800µL.**

  Note: Do not use phosphate-buffered saline (PBS) for initial dissolution of Sulfo-SMCC; the reagent does not dissolve well in buffers exceeding 50mM total salts. However, once dissolved, the solution can be further diluted in PBS or other non-amine buffers.
- 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).
- Molecules to be reacted with the maleimide moiety must have free (reduced) sulfhydryls. Reduce peptide disulfide bonds with Thermo Scientific™ Immobilized TCEP Disulfide Reducing Gel (Product No. 77712). For proteins, reduce disulfide bonds using 5mM TCEP (1:100 dilution of Thermo Scientific™ Bond-Breaker™ TCEP Solution, Product 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, Product No. 20408). Sulfhydryls can be added to molecules using *N*-succinimidyl S-acetylthioacetate (SATA, Product No. 26102) or 2-iminothiolane•HCl (Traut's Reagent, Product 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.

A. Material Preparation

- Conjugation Buffer: phosphate-buffered saline (PBS = 100mM sodium phosphate, 150mM sodium chloride, pH 7.2; e.g., Product No. 28372) or other amine- and sulfhydryl-free buffer at pH 6.5-7.5 (see Important Product Information) adding EDTA to 1-5mM helps to chelate divalent metals, thereby reducing disulfide formation in the sulfhydryl-containing protein
- Desalting column to separate modified protein from excess crosslinker and reaction byproducts (e.g., Zeba Spin Desalting Columns)
- Amine-containing (Protein-NH_2) and sulfhydryl-containing proteins (Protein-SH) to be conjugated

B. Protocol

Note: For best results, ensure that Protein-SH is prepared and ready to combine with Protein-NH_2 in step 5.

1. Prepare Protein-NH_2 in Conjugation Buffer.
2. Add the appropriate amount of crosslinker to the protein solution. The concentration of the Protein-NH_2 determines the crosslinker molar excess to use. Suggested crosslinker molar excesses are as follows (also see Table 1):
   - Protein samples < 1mg/mL use 40-80-fold molar excess.
   - Protein samples of 1-4mg/mL use 20-fold molar excess.
   - Protein samples of 5-10mg/mL use 5- to 10-fold molar excess.

Table 1. Crosslinker preparation and molar excess to use for 1mL of sample. Immediately before use, dissolve crosslinker in the appropriate solvent at the concentration denoted in parentheses; then add the listed volume to a 1mL protein sample. For example, to use the Thermo Scientific™ No-Weigh™ Sulfo-SMCC, dissolve the 2mg contents of the vial in 200µL of buffer and then add the prescribed volume to a 1mL sample. For the other products, the appropriate amount of dry reagent must be weighed on a balance (e.g., 2.4mg Sulfo-SMCC for dissolution in 500µL buffer).

Protein-NH_2 Concentration (based on a 50kDa protein)	**10mg/mL**	**1mg/mL**	**0.5mg/mL**
Crosslinker Molar Excess	5X	20X	50X
Sulfo-SMCC (in 50mM sodium phosphate or water)	100µL (4.8mg/mL*)	40µL (4.8mg/mL*)	50µL (4.8mg/mL*)
No-Weigh Sulfo-SMCC (in 50mM sodium phosphate or water)	50µL (10mg/mL*)	20µL (10mg/mL*)	25µL (10mg/mL*)
SMCC (in DMSO or DMF)	100µL (3.7mg/mL*)	100µL (1.5mg/mL*)	100µL (1.8mg/mL*)

*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.
5. Combine and mix 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. 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.

Additional Information

A. Please visit the Thermo Fisher website for additional information including the following item:

- Tech Tip: Attach an antibody onto glass, silica or quartz surface

B. Two-step reaction scheme

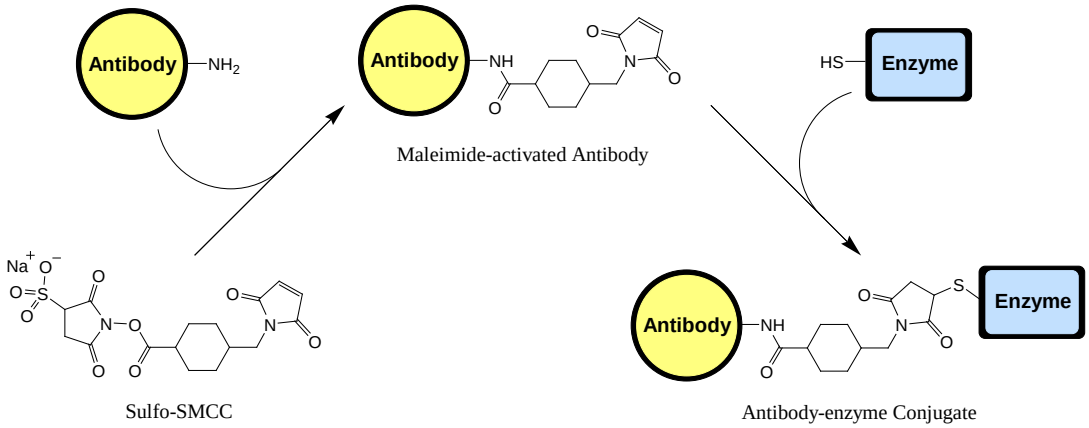

Figure 1. Two-step reaction scheme for conjugating antibody and enzyme proteins with Sulfo-SMCC. In this example, the crosslinker is first reacted with the antibody to produce a maleimide-activated protein. After excess non-reacted crosslinker and by-products are removed, the maleimide-activated antibody 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.

Related Thermo Scientific Products

Non-cleavable NHS/Maleimide Crosslinkers.

Crosslinker Name	Spacer Arm Length (Å)	Spacer Arm Composition (between ester and maleimide)	Product No. (NHS)	Product 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
$SM(PEG)_2$	17.6	Polyethylene Glycol	22102	NA
$SM(PEG)_4$	24.6	Polyethylene Glycol	22104	NA
$SM(PEG)_6$	32.5	Polyethylene Glycol	22105	NA
$SM(PEG)_8$	39.2	Polyethylene Glycol	22108	NA
$SM(PEG)_{12}$	53.4	Polyethylene Glycol	22112	NA
$SM(PEG)_{24}$	95.2	Polyethylene Glycol	22114	NA

31007 **Maleimide-Activated NeutrAvidin™ Protein**, 5mg

31485 **EZ-Link™ Maleimide-Activated Horseradish Peroxidase,** 5mg

77606 **Imject™ Maleimide Activated Mariculture Keyhole Limpet Hemocyanin (mcKLH),** 2mg

77116 **Imject™ Maleimide Activated Bovine Serum Albumin,** 2mg

Cited and Other General References

1. Ishikawa, E., *et al.* (1983). Enzyme-labeling of antibodies. *J Immunoassay* **4:**209-327.
2. Brinkley, M.A. (1992). A survey of methods for preparing protein conjugates with dyes, haptens and cross-linking reagents. *Bioconjugate Chem* **3:**2-13.
3. Hashida, S., *et al.* (1984). More useful maleimide compounds for the conjugation of Fab to horseradish peroxidase through thiol groups in the hinge. *J Appl Biochem* **6:**56-63.
4. Mattson, G., *et al.* (1993). A practical approach to cross-linking. *Molecular Biology Reports* **17:**167-83.
5. Partis, M.D., *et al.* (1983). Cross-linking of proteins by omega-maleimido alkanoyl *N*-hydroxysuccinimide esters. *J Protein Chem* **2:**263-77.
6. Yoshitake, S., *et al.* (1982). Mild and efficient conjugation of rabbit Fab and horseradish peroxidase using a maleimide compound and its use for enzyme immunoassay. *J Biochem* **92:**1413-24.

Product References

Bonacci, T.M., *et al.* (2005). Regulatory interactions between the amino terminus of G-protein βγ subunits and the catalytic domain of phospholipase Cβ2. *J Biol Chem* **280:**10174-81.

Mamedova, A.A., *et al.* (2004). Substrate-induced conformational change in bacterial complex I. *J Biol Chem* **279:**23830-6.

Medina, R., *et al.* (2004). The hydrodynamic properties of dark- and light-activated states of *n*-Dodecyl ß-D-maltoside-solubilized bovine rhodopsin support the dimeric structure of both conformations. *J Biol Chem* **279:**39565-73.

Rodriguez, P. *et al*. (2004). Critical evaluation of cardiac Ca^2+-ATPase phosphorylation on serine 38 using a phosphorylation site-specific antibody. *J Biol Chem* **279:**17111-19.

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