INSTRUCTIONS

Thermo SCIENTIFIC

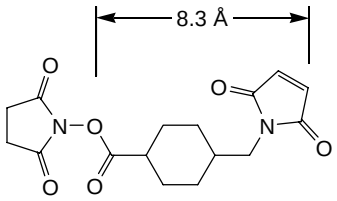
SMCC and Sulfo-SMCC

22122 22360 22322 22622

0581.8

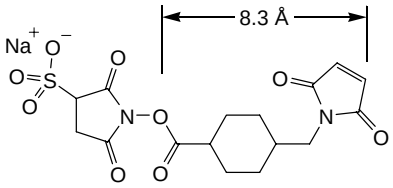
Number	Description
22360	**SMCC** (succinimidyl 4-[*N*-maleimidomethyl]cyclohexane-1-carboxylate), 50 mg

Molecular Weight: 334.32
Spacer Arm: 8.3 Å
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), 1 g
22322	**Sulfo-SMCC,** 50 mg
22622	**Sulfo-SMCC, No-Weigh™ Format,** 8 × 2 mg microtubes

Molecular Weight: 436.37
Spacer Arm: 8.3 Å
Net Mass Added: 219.09
CAS #: 92921-24-9
Storage: Upon receipt store desiccated at -20° C.
Product is shipped at ambient temperature.



Introduction

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 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 10 mM, 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.
- No-Weigh Microtube Handling: Immediately before use, puncture the microtube foil with a pipette tip, add 200 µl of buffer or ultrapure water and pipette up and down to mix. After use, cut the used microtube from the microtube strip and discard. Store the unused microtubes in the foil pouch provided.
- 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 Immobilized TCEP Disulfide Reducing Gel (Product No. 77712). For proteins, reduce disulfide bonds using 5 mM TCEP (1:100 dilution of Bond-Breaker® TCEP Solution, Product No. 77720) for 30 minutes at room temperature, followed by two passes through a suitable desalting column (e.g., Zeba™ Desalt Spin 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, 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 1-5 mM 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 Desalt Spin 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 < 1 mg/ml use 40-80-fold molar excess.
    - Protein samples of 1-4 mg/ml use 20-fold molar excess.
    - Protein samples of 5-10 mg/ml use 5- to 10-fold molar excess.

Table 1. Crosslinker preparation and molar excess to use for 1 ml of sample. Immediately before use, dissolve crosslinker in the appropriate solvent at the concentration denoted in parentheses; then add the listed volume to a 1 ml protein sample. For example, to use the No-Weigh Sulfo-SMCC, dissolve the 2 mg contents of the microtube in 200 µl of buffer and then add the prescribed volume to per 1 ml sample. For the other products, 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).

Protein-NH_2 Concentration (based on a 50 kDa protein)	**10 mg/ml**	**1 mg/ml**	**0.5 mg/ml**
Crosslinker Molar Excess	5X	20X	50X
Sulfo-SMCC (in buffer)	100 µl (4.8 mg/ml*)	40 µl (4.8 mg/ml*)	50 µl (4.8 mg/ml*)
No-Weigh Sulfo-SMCC (in buffer)	50 µl (10 mg/ml*)	20 µl (10 mg/ml*)	25 µl (10 mg/ml*)
SMCC (in DMSO or DMF)	100 µl (3.7 mg/ml*)	100 µl (1.5 mg/ml*)	100 µl (1.8 mg/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 Pierce website for additional information including the following item:

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

B. Two-step reaction scheme

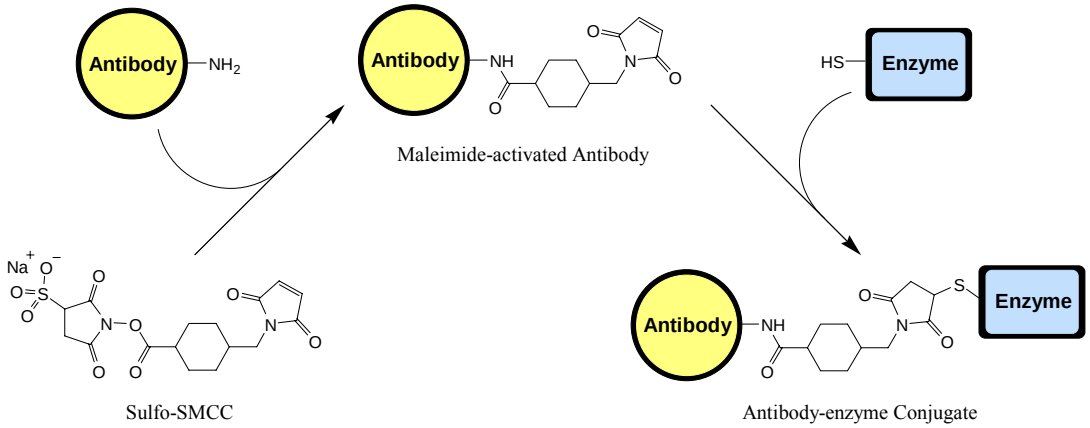

Figure 1. Two-step reaction scheme for conjugating antibody and enzyme proteins with Sulfo-SMCC. In this example, the crosslinker is first reacted with the antibody to produce a maleimide-activated protein. After excess non-reacted crosslinker and by-products are removed, the maleimide-activated antibody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

Noncleavable NHS/Maleimide Pierce 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

31007 **EZ-Link® Maleimide Activated NeutrAvidin™ Protein**, 5 mg

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

31486 **EZ-Link Maleimide Activated Alkaline Phosphatase,** 5 mg

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

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

89889 **Zeba Desalt Spin Columns,** 5 × 2 ml columns for desalting 200-700 µl samples

89891 **Zeba Desalt Spin Columns,** 5 × 5 ml columns for desalting 500-2,000 µl samples

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.