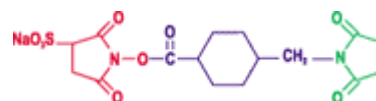


SMCC, sSMCC

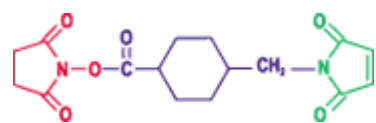
Heterobifunctional cross-linkers

Product Description

Catalog number: UP17412A, 100mg UP17412B, 50mg
Name: **Sulfo-SMCC**
Formula: 4-(N-maleimidomethyl)cyclohexane-1-carboxylic 3-sulfo-n-hydroxysuccinimide ester
 CAS: 92921-24-9 , C₁₆H₁₇N₂O₉SNa, M.W.= 436.3



Catalog number: UP34253A, 50mg UP34253B, 100mg
Name: **SMCC**
Formula: Succinimidyl-4-(N-maleimidomethyl)cyclohexane-1-carboxylate
 CAS: 64987-85-5 , C₁₆H₁₈N₂O₆, M.W.= 334.3



Storage : -20°C (possible at +4°C) (U), protect from moisture and light.

General Considerations

Cross-linkers are chemical reagents used to conjugate molecules together by a covalent bond. Several atoms separate the 2 molecules, forming the 'spacer arm'. The conjugate associates the characteristics and biological activities of each component.

Cross linkers have become important tools for the preparation of conjugates used in a lot of immunotechnologies, and for protein studies (structure, interactions, activity, degradation...). To that point, heterobifunctional crosslinkers are probably the most interesting, because they present 2 reactivities that allow the conjugation of molecules in a defined manner, avoiding notably the formation of dimers and polymers. The choice of reactivities is determinant to the design of the right conjugate. Considering the final result, an important other thing is the nature and length of the spacer. The SMCC contains the 2 reactivities toward amines, through the succinimide group, and a reactivity toward thiols, through the maleimide group.

Uptima offers a high quality SMCC to answer the needs of coupling proteins and peptides for biological and immunoassays like (other crosslinkers are available):

- Obtention of immunogen carrier-hapten
- Obtention of labeled affine probes: for example, antibodies coupled to enzyme for immunoblotting, fluorophore-peptides conjugates for the study of receptors, enzyme-drugs for using as tracers in ELISA...
- Obtention of oligomeric conjugates : conjugates of oriented peptides for immunization, dimeric proteins for structural studies, grafting haptens onto cells...
- Obtention of biologically active conjugates: specific antibody coupled to drugs for immunotargeting techniques, immunotoxins, ...

Ask for other crosslinkers similar to SMCC, i.e. SICC ([U1469](#)), or other with different spacer natures and lengths that are available from Uptima.

Scientific and Technical Information

- Open the vial when it has reached room temperature only.
- The chemical group **N-hydroxysuccinimydyl** (NHS) reacts in aqueous phase, optimally at neutral pH (7.5 (6.5-8.5) or higher, on primary ($-NH_2$) and secondary amines ($=NH$) (in fact on its deprotonated form): amines present in proteins (Lys aminoacid) and in a lower proportion on NH_2 located in terminal peptidic chains. The reaction competes with hydrolysis, that increases with pH, and with the high dilutions of the molecule that should be derivatized. The reaction is completed usually within 1-2hours (check absorbance at 260nm do not increase anymore).
- The **sulfonyl** moiety ($NaSO_3$) introduces a hydrophilic group, that allows the product not to cross biological membranes. This is particularly useful to modify, in situ on cells, proteins presented outside membranes, and if one wants to avoid the modification of intracellular proteins that may affect further analysis. An other interest of the sulfonyl group is to permit the solubilization of the product directly in aqueous buffers, up to 10mM, avoiding the use of organic solvents like DMSO or DMF, that are possibly nocive to cells or applications.
- The **spacer** arm measures 11.5 Angstroms length. It contains an aromatic cycle not linked directly, that stabilizes the maleimide reactivity.
- The **maleimide** group reacts very specifically with sulfhydryls $-SH$ at neutral pH 6.5-7.5. The reaction is rapid (a few minutes for cystein), but may require 1-2 hours to be completed in certain conditions. The competitive hydrolysis forming maleamic acid becomes noticeable when pH go up 8.0, where the reactivity with amines begins to be possible. It is stable in 0.1 M phosphate, pH 7.0, 4 °C, for 64 h ([Yoshitake 1979](#)). In usual conditions, one should start with a ratio of 10-20 moles of maleimide per mole of protein. With SH-peptides, a molar 1:1 incubation ratio allows almost 1:1 coupling.

Use

Protocole 1 : Conjugating an antibody with an enzyme, Peroxidase or alkaline Phosphatase

This standard protocole can be applied to polyclonal and monoclonal purified antibodies.

- 1- Dialyse the antibody at 10 mg/ml in PBS (NaCl 150mM, phosphate 20mM pH7.5) 4mM EDTA
- 2- Add 10mM of DTT (#UP284250) or TCEP (#UP242210), incubate 1H at +37°C
- 3- Desalt the antibody by gelfiltration with degassed PBS buffer to elute. The desalted antibody can be monitored in eluted fractions by measuring absorbance at 280nm, or a protein assay. SH concentration can be dosed by the DTNB (#UP01566) method. Use the antibody rapidly because SH oxidizes easily in contact of air; or else, keep it at +4°C if possible under nitrogen.
- 4- Dialyse the enzyme at 10mg/ml in PBS. The buffer should be free of amines (no Tris)
- 5- Add 2.5 mg of sSMCC per ml of enzyme while mixing, and incubate for 15min at +37°C. Protect from light.
Rem: non sulfonated SMCC should be added as a DMSO solution
- 6- Desalt the maleimide activated enzyme by gelfiltration in PBS. Fractions containing the enzyme can be identified by absorbance measurement at 280nm, or any other means (Coo Assay #UPF8640A, addition of substrate). Use this activated enzyme rapidly.
- 7- Add the reduced antibody to the activated enzymes, and incubate for 30min at room temperature, protected from light.
- 8- Desalt the conjugate by gelfiltration in PBS (peroxidase) or TBS (Tris 10mM NaCl 150mM pH7.4, 1mM $MgCl_2$) for the alkaline phosphatase.
- 9- Store the conjugate at +4°C with preservatives and 20% glycerol.

The immuno-conjugate can be titrated by ELISA on a coating of relevant antigen that is recognized by the antibody, and with a suitable substrate (pNPP #UP664790 for the alkaline phosphatase; TMB #UP664780 for the peroxidase).

This protocole can be adapted to other proteins than antibodies and enzymes. A set up is generally necessary for each application. It is important to check that the molecules to be coupled are pure enough. One should contain amines, the other sulfhydryls. Sulfhydryls are rarely naturally present, but generated either by reduction like in the protocole 1, or by chemical modification of amines with SATA #UP84235A, or Iminothiolane #UP42425A reagent.

Contact your local distributor

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Protocole 2: Conjugating a Cys-peptide to a protein (anticorps, carrier...)

Peptides are frequently synthesized with a terminal cysteine in terminal positions, to facilitate their attachment to other molecules. One can adapt the protocole 1 by substituting the antibody for the peptide and the enzyme for the protein.

Rem : The cysteine (Cys-SH) of lyophilized peptides oxidizes readily to the air, forming dimeric peptides (with disulfide bridges –S-S–), and impairing the right conjugation. The concentration of –SH can be quantified by the DTNB (UP01566H) method. If the –SH level was insufficient, the reduction then desalting steps are naturally not useful.

Rem : Uptima offers optimised carriers, MaxiBind™ to prepare peptides-conjugates for immunization and screening purposes. Ask for them!

Other Information

For use *in vitro* only, not for diagnostic.

For any information, please contact Uptima, or your local distributor.

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Literature

Maham, D.G., et.al. (1987) *Anal. Biochem.* 162, 163-170.

Yoshitake, S., et.al. (1979) *Eur. J. Biochem.* 101, 395-399.

Hashida, S., et .al. (1984) *J. Applied Biochem.* 6, 56-63.

Samoszuk, M.K., et.al. (1989) *Antibody Immunocon. Radiopharm.* 2(1), 37-46.

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