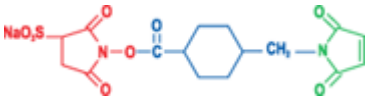
FT-17412A

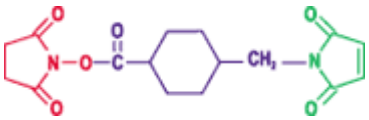
SMCC, sSMCC, lc-SMCC Heterobifunctionnal crosslinkers

Products Description

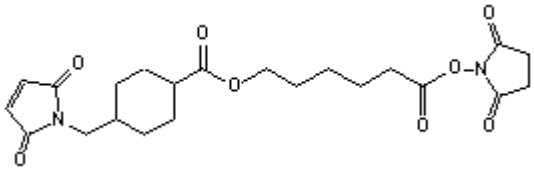
Catalog number: UP17412A, 100mg UP17412B, 50mg
Name: **Sulfo-SMCC**
Formula : 4-(N-maleimidomethyl)cyclohexane-1-carboxylic 3-sulfo-n-hydroxysuccinimide ester [Syn.: sSMCC, Water-Soluble SMCC]
CAS: 92921-24-9 , **MW= 436.3**



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



Catalog number: L7739A, 50mg L7739B, 100mg L7739, 250mg
Name: **LC-SMCC**
Formula : Succinimidyl-4-(N-maleimidomethyl)cyclohexane-1-carboxy-(3-AmidoCaproate) [syn.: Long-Chain SMCC]
CAS: 64987-85-5 , **MW= 447.5**
Spacer is 16.1A long (16 atoms)



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

General Considerations

SMCC is an popular heterobifunctional crosslinking agent. It contains 2 reactivities that allow the conjugation of molecules in a defined manner, avoiding notably the formation of dimers and polymers:
. a succinimidyl ester group, that reacts on target amines of a moleucle1 (a protein, or an amino-nucleotide)
. a maleimidyl group, that reactos on target sulfhydryl group of molecule2 (i.e. a reduce protein or Cys-peptides)

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

- Preparation of immunogens carrier-hapten
- Preparation 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…
- Preparation of oligomeric conjugates: conjugates of oriented peptides for immunization, dimeric proteins for structural studies, grafting haptens onto cells…
- Preparation of biologically active conjugates: specific antibody coupled to drugs for immunotargetting techniques, immunotoxins, …

Ask for other crosslinkers similar to SMCC that are available from Uptima (see related products).
MAL-PEO$_4$-NHS #AL6580 replace advantageously (sulfo)SMCC in most applications thanks it hydrophilic spacer conferring benefits such as improved activity and stability of the conjugate, non immunogenicity, ...

Scientific and technical Information

- The chemical group **N-hydroxysuccinimidyl** (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 that absorbance at 260nm does not increase anymore).
- The **sulfonyl** moeity (NaSO3) 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, >10mM in several buffers pH5-7.5, and up to 10mg of sSMCC/ml at room temperature. At the opposite, for SMCC, organic solvant as DMF DMSO should be added for solubilization, but this may be detrimental to protein activity or toxic to cells. In such applications, sulfo derivative is preferable.
- The **spacer** arm of SMCC measures 9.3 Angstroms length. It contains an aromatic cycle not linked directly, that stabilizes the maleimide reactivity. LC-SMMC spacer arm measures 16.1A.
- The **maleimide** group reacts very specifically with sulfhydryls –SH at neutral pH 6.5-7.5. The reaction is rapid (a few minutes for cysteine), 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. Additionnal information on inquire
- A study of the hydrolysis rate of the maleimide group showed Sulfo-SMCC is less prone to hydrolysis (no decomposition at pH7 at 30°C within 6 hours) than the non-sulfonated SMCC.
- After conjugation, net added mass is 219.09gm/mol for SMCC (and for Sulfo-SMCC).
- **Applications**: SMCC has become very popular in preparing immunoreagents, and in particular, with HRP and AP enzymes. It is most useful conjugating reduced IgG and F(ab')2 fragments, but this an be applied as well as to native antibodies modified by SATA or Iminothiolane to introduced SH groups.
  Typically the protein1 (enzyme) is first activated by SMCC, through its amino groups reacting specifically with the NHS group of the crosslinker. Then coupled the activated protein1 is reacted with the protein2 containing SH groups (reduced antibody) through the maleimide group. The 2-steps methods is superior to glutaraldehyde and metaperiodate methods of enzyme conjugation.

Directions for use

Open the vial when it has reached room temperature.

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

This standard protocol can be applied to polyclonal and monoclonal purified antibodies.(r)

1- Dialyze 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 disgazed 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- Dialyze 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 protocol 1, or by chemical modification of amines with SATA #UP84235A, or Iminothiolane #UP42425A reagent.

Protocol 2: Conjugating a Cys-peptide to a protein (antibody, carrier…)

Peptides are frequently synthesized with a terminal cysteine (Cys) in terminal position, to facilitate their attachment to other molecules. One can then adapt the protocol 1 by substituting the antibody by the peptide and the enzyme by the protein to be conjugated.

Rem : The cysteine should display a free thiol group (-SH) on the lyophilized peptide. The thiols however oxidizes readily to the air, forming dimeric peptides (with disulfide bridges –S-S-), and impairing the conjugation. The concentration of –SH can be quantified by the DTNB (UP01566H) method. If the –SH level was insufficient, a reduction then desalting steps are required.
Rem : Uptima offers optimized carriers, MaxiBind™ to prepare peptides-conjugates for immunization and screening purposes. Ask for them!

References

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.

Other information

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

Related / associated products and documents

See BioSciences Innovations catalogue and e-search tool.
-Desalting tools (CelluSep dialysis, desalting columns)
-Other crosslinkers, i.e. other heterobifunctional crosslinkers with NHS and MAL reactivities:

- MAL-PEOx-NHS #AL6580 (hydrophilic spacer)
- SMCC Plus #JQ3870
- EMCS UP19548A& Sulfo-EMCS #UPL7729A
- GMBS #UP49608A & Sulfo-GMBS #UP52444A
- MBS #UP21608A
- SMPB #UP28072A & Sulfo-SMPB #UP52757A
- SICC #U1469
- SMCC-hydrazide #BI1281

-Useful modifiers and other conjugation technologies:

- SATA #84235A, Iminothiolane #42425A
- SMCC-hydrazide #BI1281

- Hydralink Conjugation kit #BL1501 and -SMCC activated proteins
- KLH #86734A, BSA #86695A, OVA #23066A
- crosslinkers (SANH #BL9270, MHPH #BL9401)
- • B-PE #QU5890, R-PE #QU5900, APC #QU5880,

Catalog size quantities and prices may be found at http://www.interchim.com.
For any information, please ask : Uptima / Interchim; Hotline : +33(0)4 70 03 73 06

Disclaimer : Materials from Uptima are sold **for research use only**, and are not intended for food, drug, household, or cosmetic use.
Uptima is not liable for any damage resulting from handling or contact with this product. Rev.U01E-H09E-C02E