FT-28067A

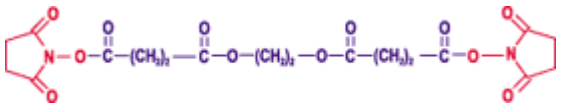
EGS, Sulfo-EGS

Homobifunctionnal alk.cleavable crosslinkers

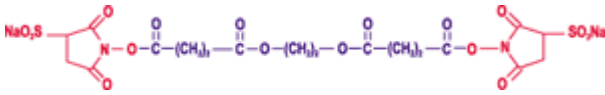
Features

Mild alkaline cleavable homobifunctionnal amine reactive crosslinker

Catalog number: UP28067A, 2g
Name: **EGS**
Formula : EthylGlycol bis(SuccinimidylSuccinate)
M.W.= 456.37, CAS: 70539-42-3



Catalog number: UP24455A, 100mg UP24455B, 50mg
Name: **Sulfo-EGS**
Formula : EthylGlycol bis(SulfoSuccinimidylSuccinate)
M.W.= 660.45



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

Introduction

Cross-linkers are chemical reagents used to conjugate molecules together by a covallent bound. 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…). **Homobifunctionnal** cross-linkers present 2 identic reactivities. The choice of the reactivities is determinant to the design of the right conjugate. EGS crosslinker react toward amines, through the succinimide group, and contain a **alkaline cleavable** linkage.

Uptima offers a high quality EGS and it's sulfonated form to answer the needs of coupling proteins and peptides for biological studies and immunoassays like (other cross-linkers are available): (see literature below)

- Obtention of conjugates for structural studies (receptors, ligand interactions…)
- Reticulation of proteins (complexes) in solution or immobilized (receptors)
- Obtention of oligomeric conjugates : poly- peptides
- Immobilization on polystyrene or glass surfaces for immunoassays and biosensors
- Graffting peptides onto gels for chromatography separations
- Graffting haptens onto cells and particules (beads) for diagnostics…

Scientific and technical Information

- The chemical group **N-hydroxysuccinimydyl** (**NHS**) reacts in aqueous phase on primary ($-NH_2$) and secondary amines (=NH) (in fact on its deprotonated form), optimally at neutral pH or higher : amines present in proteins (Lys aminoacid) and in a lower proportion on NH_2 located in terminal peptidic chains. The reaction occurs in few minutes in organic media at room temperature. 30min to 2Hr (typically 1Hr) reaction at room temperature in aqueous buffers but in competition with hydrolysis, that increases with pH, and with the high dilutions of the molecule that should be coupled. The reaction with amines occurs typically at pH6.5-8.5 in 1hour. n-HydroxySuccinimide is released and should usually be removed before use of the conjugate.

- The **sulfonyl moeity** (**$NaSO_3$**) introduces a negatively charged hydrophilic group, that allows the product not to cross biological membranes. It reacts out-side cells, when EGS reacts also in the the membrane and in the cells. An other interest of the sulfonyl group is to permit the solubilisation of the product directly in aqueous buffers, avoiding the use of organic solvants like DMSO or DMF, which are possibly nocive to cells or applications.

- EGS is **soluble** in acetic acid/water (1:1) up to 50 mg/ml and in DMF. Sulfo-EGS is soluble up 10mM in water or buffer (citrate pH5)(a small amount of DMF or DMSO increase solubility). Unused solutions should not be re-used. Sulfo-EGS can be added directly to the sample.

- The **spacer arm** of EGS measures 16.1 Angstroms lenght. It is stable, allowing to prepare and use the conjugate. It can however be cleaved in mild alkaline conditions, i.e. with 0.2-1M hydroxylamine at pH8.5.

- Examples of protocols are given in the literrature. As guidelines, here are some information:

Coupling of proteins in solution: the molecule(s) to be coupled is (are) prepared in PBS (20mM phosphate, 150mM NaCl, pH7.5). Other suitable buffers include HEPES, carbonate and borate (but not Tris) provided the pH is kept between 7 and 9. Crosslinker is added at 5 to 40 molar excess over the protein. Incubation may last 30min to 1H incubation at room temperature (or 1-2H at +4°C if thermolabile proteins).The crosslinking leads to conjugates (dimers…) and to reticulated forms. If different molecules are mixed, homo and hetero conjugates are obtained.

Cell crosslinking: the cells at 1-10% suspension, mixed with the protein to be coupled at 2-10nM, are incubated with 0.5-4mM of crosslinker. +4°C is recommended for many cells, and agitation should be mild but continuous.

Immobilization of proteins: the soluble protein is incubated with the crosslinker, then on the aminated (or protein-coated) support (polystyrene microplate, agarose gel…).. The concentration of protein and crosslinkers should be determined depending on protein nature and coating density. As starting concentations, crosslinker is used at 1-5mM with the protein of interest at 1-5mM.

If a precipitate is observed, protein and crosslinker concentrations should be decreased, or DMSO added up to 20% final concentration in the reaction mixture.

A stop reaction may be usefull, for example with 20mM Lysine or with a Tris buffer during 15-30min.
Prepare extemporaneously a 2M Hydroxylamine solution in phosphate pH8.5.
The reaction is advantadgely performed at +37°C.

A separation technic is usually necessary to isolate conjugates (gelfiltation, dialysis, cell washing…)

Other information regarding NHS reactivity are available (NT-NHS: buffers, conditions of use…).

Literature: EGS

Abdella, P.M., et al.; A new cleavable reagent for cross-linking and reversible immobilization of proteins. Biochem. Biophys. Res. Commun. (1979), 87, 734-742
Browning, J. and Ribolini, A.; Studies on the differing effects of tumor necrosis factor and lymphotoxin on the growth of several human tumor lines. J. Immunol. (1989), 143, 1859-1867.
Massague, J., et al.; Affinity labeling of multiplication stimulating activity receptors in membranes from rat and human tissues. J. Biol. Chem. (1981), 256, 2122-2125
Millar, J.B. and Rozengur, E.; Chronic desensitization to bombesin by progressive down-regulation of bombesin receptors in Swiss 3T3 cells. J. Biol. Chem. (1990), 265, 12052-12058.
Biol. Chem. 261, 205-210.

Other information

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

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

See BioSciences Innovations catalogue and e-search tool.

-Desalting tools (CelluSep dialysis, desalting columns)

-Other cleavable homobifunctionnal amine reactive crosslinkers: • DSP UP18971 (thiol cleavable), DST UP28068 (oxidizer cleavable)

-Homobifunctionnal amine reactive crosslinkers (non cleavable): • BS3 and NHS-PEO-NHS , DTPB #18628A

-Heterobifunctional crosslinkers: • MAL-PEOx-NHS #AL6580 (hydrophilic spacer)
• SMPB #UP28072A & Sulfo-SMPB #UP52757A • SMCC-hydrazide #BI1281

-Other conjugation technologies: • Hydralink Conjugation kit #BL1501 and crosslinkers (SANH #BL9270, MHPH #BL9401)

-Useful modifiers: • SATA #84235A, Iminothiolane #42425A

Catalog size quantities and prices may be found at http://www.interchim.com.
Please inquire for higher quantities (availability, shipment conditions).