

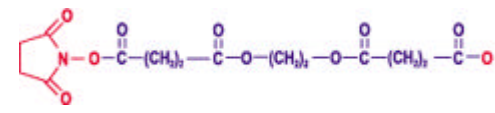
# EGS, Sulfo-EGS

## Homobifunctional alk.cleavable cross-linkers

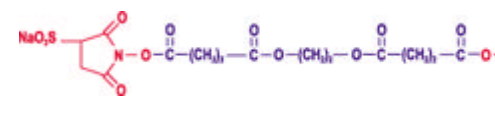
### Product Description

Mild alkaline cleavable homobifunctional amine reactive crosslinker

**Catalog number:** UP28067A, 2g  
**Name:** EGS  
**Formula :** EthylGlycol bis(SuccinimidylSuccinate)  
 $C_{18}H_{20}N_2O_{12}$ , CAS: 70539-42-3 , **M.W.= 456.37**



**Catalog number:** UP24455A, 100mg      UP24455B, 50mg  
**Name:** Sulfo-EGS  
**Formula :** EthylGlycol bis(SulfoSuccinimidylSuccinate)  
 $C_{18}H_{18}N_2O_{11}S_2Na_2$ ,      **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 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...). **Homobifunctional** cross-linkers present 2 identical reactivities. The choice of the reactivities is determinant to the design of the right conjugate. EGS crosslinker reacts toward amines, through the succinimide group, and contains an **alkaline cleavable** linkage.

Uptima offers a high quality EGS and its 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
- Grafting peptides onto gels for chromatography separations
- Grafting haptens onto cells and particles (beads) for diagnostics...

### Scientific and Technical Information

- The chemical group N-hydroxysuccinimide (NHS) reacts in aqueous phase on primary (–NH<sub>2</sub>) and secondary amines (=NH) (in fact on its deprotonated form), optimally at neutral pH or higher : amines present in proteins (Lys amino acid) and in a lower proportion on NH<sub>2</sub> located in terminal peptidic chains. The reaction occurs in few minutes in organic media at room temperature, and also 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 pH 6.5-8.5 in 1 hour. n-Hydroxysuccinimide is released and should usually be removed before use of the conjugate.
- The sulfonyl moiety (NaSO<sub>3</sub>) introduces a negatively charged hydrophilic group, that allows the product not to cross biological membranes. It reacts outside cells, when EGS reacts also in the membrane and in the cells. Another interest of the sulfonyl group is to permit the solubilisation of the product directly in aqueous buffers, avoiding the use of organic solvents 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 to 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.

FT-UP28067

- The spacer arm of EGS measures 16.1 Angstroms length. 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.

Prepare extemporaneously a 2M Hydroxylamine solution in phosphate pH8.5.

The reaction is advantageously performed at +37°C.

- Examples of protocols are given in the literature. 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.

**Immobilization of proteins:** a soluble protein is conjugated to an aminated (or protein-coated) support (microplate, gel...). Crosslinker is used at 1-5mM with the protein of interest at 1-5mM.

**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 protein is incubated with the crosslinker on the desired protein that is coated on polystyrene or other support. The concentration of protein and crosslinkers should be determined depending on protein nature and coating density.

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 useful, for example with 20mM Lysine or with a Tris buffer during 15-30min.

A separation technique is usually necessary to isolate conjugates (gel filtration, dialysis, cell washing...)

Other information regarding NHS reactivity are available ([NT-NHS](#); buffers, conditions of use...).

## 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 :

CelluSep™ : dialysis membranes

Other cleavable homobifunctional amine reactive crosslinkers: DSP [UP18971](#) and DTPB [UP99796](#) (thiol cleavable), DST [UP28068](#) (oxidizer cleavable), EGS [UP280067](#) (mild alkaline cleavable)...

### 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.

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