INSTRUCTIONS

EGS

Sulfo-EGS

21565 21566

0545.1

Number	Description
21565	**EGS** [Ethylene glycolbis(succinimidylsuccinate)], 1 g Molecular Weight: 456.36 Spacer Arm: 16.1 Å **Storage:** Upon receipt store desiccated at 4°C. Product is shipped at ambient temperature.
21566	**Sulfo-EGS** [Ethylene glycolbis(sulfosuccinimidylsuccinate)], 50 mg Molecular Weight: 660.45 Spacer Arm: 16.1 Å **Storage:** Upon receipt store desiccated at -20°C. Product is shipped at ambient temperature.

Introduction

EGS is a water-insoluble, homobifunctional *N*-hydroxysuccinimide ester (NHS ester) and Sulfo-EGS is its water-soluble analog. The spacer arm contains two cleavable ester sites that may be broken with hydroxylamine, which yields two fragments with terminal amide bonds and the release of ethylene glycol. These reagents are often used for conjugating radiolabeled ligands to cell surface receptors. The water insoluble reagent EGS does not possess a charged group, is lipophilic and, therefore, membrane-permeable and useful for intracellular and intramembrane protein conjugation. EGS is first dissolved in DMSO or DMF and added to the aqueous reaction mixture at a final solvent concentration of 10-20%, to minimize detrimental affects to the protein.

Accessible α-amine groups present on the N-termini of proteins and peptides and ε-amine of lysine react with NHS esters at pH 7-9 to form covalent amide bonds. The reaction results in the release of *N*-hydroxysuccinimide. Hydrolysis of the NHS ester is the major competing reaction and increases with increasing pH and occurs more readily in dilute protein solutions. NHS ester cross-linking reactions are most commonly performed in phosphate, carbonate/bicarbonate, HEPES and borate buffers. Other buffers may also be used provided they do not contain primary amines such as Tris or glycine. Using a large excess of Tris or glycine at neutral-to-basic pH can quench the reaction.

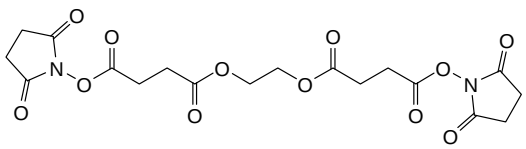

Figure 1. Molecular structure of EGS.

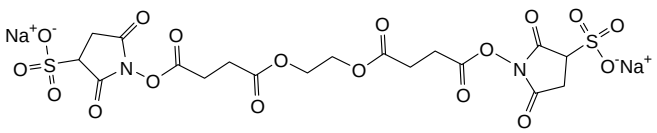

Figure 2. Molecular structure of Sulfo-EGS.

Important Product Information

- EGS and Sulfo-EGS are moisture-sensitive. Store desiccated at 4-8°C. Equilibrate vial to room temperature before opening to avoid moisture condensation onto the product.
- Prepare these cross-linkers immediately before use. The NHS ester moiety readily hydrolyzes and becomes non-reactive; therefore, stock solutions must not be prepared for storage. Discard any unused reconstituted cross-linker. DMSO or DMF are hygroscopic and absorb water, which promotes hydrolysis.
- Avoid buffers containing primary amines (e.g., Tris or glycine) during conjugation as they will react with the NHS ester and will inhibit and reduce conjugation efficiency of the intended molecules.

- The amount of cross-linker to use for each reaction depends on the protein amount and concentration. When cross-linking dilute protein solutions (e.g., 2 mg/ml) a greater molar fold excess of cross-linker is used compared to a concentrated protein solution (e.g., 10 mg/ml). For example, use a 20- to 50-fold molar excess of reagent for a 2 mg/ml protein solution or ≥10-fold molar excess of cross-linker for a 10 mg/ml protein solution. Manipulating the molar ratio of cross-linker to the protein can control the extent of conjugation and polymerization.
- These reagents may form a microprecipitate at high concentrations when added to the aqueous medium, which results in a cloudy appearance. Nevertheless, the reaction will proceed efficiently and the microprecipitate may disappear during conjugation. The protocol may be modified to ensure complete dissolution of the NHS ester. For example, the aqueous phase may be supplemented with additional organic solvent.

Example Procedure for Cross-linking Proteins in Solution

A. Materials Required

- Cross-linker Solution: Just before use, dissolve EGS in dry DMSO at 10-25 mM. Sulfo-EGS may be dissolved in water or buffer to 10 mM.

  Note: Sulfo-EGS is soluble up to ~10 mM in water and many commonly used buffers; however solubility decreases with increasing salt concentration.
- Reaction Buffer: Phosphate Buffered Saline (e.g., BupH™ Phosphate Buffered Saline Packs containing 0.1 M phosphate, 0.15 M NaCl, pH 7.2, Product No. 28372), or other buffer at pH 7-9 may be used, such as HEPES, bicarbonate/carbonate or borate buffers, provided it does not contain primary amines.
- Quenching Solution: 1 M Tris, pH 7.5 or other amine-containing buffer

  Note: Quenching the reaction is optional and may not be required for some applications.

B. Procedure

1. Prepare the protein sample in reaction buffer.
2. Add the desired cross-linker to the protein sample. Add a 10-fold molar excess cross-linker over the protein when the protein concentration is above 5 mg/ml. If the protein concentration is below 5 mg/ml, add a 20- to 50-fold molar excess of the cross-linker. The cross-linker may be used at a final concentration of 0.25-5 mM.

   Note: To minimize detrimental affects to the protein, do not exceed 10-20% of DMSO in the final reaction volume.
3. Incubate the reaction mixture at room temperature for 30 minutes or on ice for 2 hours.
4. Quench the reaction for 15 minutes with a solution containing amines such as Tris or glycine at a final concentration of ~20-50 mM in the reaction mixture.
5. Incubate the reaction mixture for an additional 15 minutes.

Example Procedure for Intra- and Extra- cellular Protein Cross-linking

Note: For cross-linking cell surface proteins use Sulfo-EGS, as it will not the cell membrane. Use EGS when cross-linking proteins within the cell. Note that some EGS will react with amines as it traverses the cell membrane.

1. Incubate membranes (0.1-0.5 mg) for 1 hour at 4°C with ligands (5-10 nM) in a total volume of 100 µl PBS.
2. Add EGS or Sulfo-EGS solution to a final concentration of 1-2 mM.
3. Incubate the reaction mixture for 30 minutes at room temperature or 2 hours on ice.
4. Add the Quenching Solution to a final concentration of 10-20 mM and incubate for 15 minutes.

Procedure for Cleaving EGS Cross-linked Compounds with Hydroxylamine•HCl

This procedure is modified from the method used by Abdella, *et al.* (1979).

A. Materials Required

- Hydroxylamine•HCl (Product No. 26103)
- Phosphate Buffered Saline (PBS) adjusted to pH 8.5

B. Procedure

5. Prepare 2.0 M hydroxylamine•HCl by adding it to PBS and adjusting the pH back to 8.5. Prepare this solution immediately before use.
6. Warm the hydroxylamine•HCl solution quickly to 37°C and incubate equal volumes of sample and hydroxylamine solution for 3-6 hours with stirring. Alternatively, incubate for 6 hours at room temperature, although efficiency may be lower.
7. An aliquot of the hydroxylamine cleaved sample, containing 13-14 µg quantities of protein, can be examined by SDS-PAGE to determine effectiveness of the cleavage.

Additional Information

Please visit the web site for additional information relating to this product including the following items:

- Tech Tip: Protein stability and storage
- Tech Tip Protocol: Determine reactivity of NHS ester biotinylation and cross-linking reagents
- Request a free copy of Double-Agents™ Cross-linking Reagents Selection guide

Related Thermo Scientific Products

25200-25244	**Precise™ Protein Gels** (see catalog or web site for a complete listing)
24590	**GelCode® Blue Stain Reagent,** 500 ml
23225	**BCA Protein Assay Kit**

References

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Browning, J. and Ribolini, A. (1989). Studies on the differing effects of tumor necrosis factor and lymphotoxin on the growth of several human tumor lines. *J. Immunol.* **143:**1859-67.

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