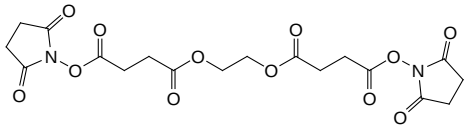
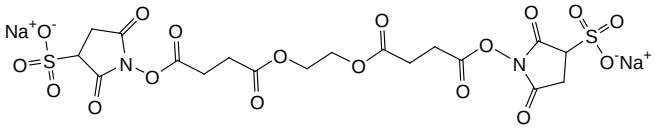
INSTRUCTIONS

EGS
Sulfo-EGS

21565 21566

0545.5

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

Introduction

Thermo Scientific™ 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 crosslinking 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.

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 crosslinkers 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 crosslinker. 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 crosslinker to use for each reaction depends on the protein amount and concentration. When crosslinking dilute protein solutions (e.g., 2mg/mL), a greater molar fold excess of crosslinker is used compared to a concentrated protein solution (e.g., 10mg/mL). For example, use a 20- to 50-fold molar excess of reagent for a 2mg/mL protein

solution or ≥ 10-fold molar excess of crosslinker for a 10mg/mL protein solution. Manipulating the molar ratio of crosslinker to the protein can control the extent of conjugation and polymerization.

- These reagents can 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 Crosslinking Proteins in Solution

A. Materials Required

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

  Note: Sulfo-EGS is soluble up to ~10mM in water and many commonly used buffers; however solubility decreases with increasing salt concentration.

- Reaction buffer: Phosphate-buffered saline (e.g., Thermo Scientific™ BupH™ Phosphate Buffered Saline Packs containing 0.1M phosphate, 0.15M 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: 1M 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 crosslinker to the protein sample. Add a 10-fold molar excess crosslinker over the protein when the protein concentration is above 5mg/mL. If the protein concentration is below 5mg/mL, add a 20- to 50-fold molar excess of the crosslinker. The crosslinker may be used at a final concentration of 0.25-5mM.

   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 ~20-50mM in the reaction mixture.
5. Incubate the reaction mixture for an additional 15 minutes.

Example Procedure for Intra- and Extra-cellular Protein Crosslinking

Crosslinking may be performed on cells in suspension or on adherent cells in culture plates. Culture media must be washed from the cells otherwise amine-containing components will quench the reaction. NHS reactions occur more rapidly with increasing pH; therefore, pH 8.0 is used in the following example so the reaction can be completed quickly.

Note: Use Sulfo-EGS for crosslinking cell surface proteins, as it will not permeate the cell membrane. Use EGS for crosslinking proteins within the cell. Note that some EGS will react with amines as it traverses the cell membrane.

1. Prepare Crosslinker Solution, Reaction Buffer and Quenching Solution as described in the procedure above.
2. Suspend cells at ~25×10^6 cells/mL in PBS (pH 8.0).
3. Wash cells three times with ice-cold PBS (pH 8.0) to remove amine-containing culture media and proteins from the cells.

   Note: For cell-surface interaction studies, add ligands to the cells and incubate for 1 hour at 4°C.
4. Add EGS or Sulfo-EGS solution to a final concentration of 1-5mM.
5. Incubate the reaction mixture for 30 minutes at room temperature or 2 hours on ice.
6. Add the Quenching Solution to a final concentration of 10-20mM and incubate for 15 minutes.

Procedure for Cleaving EGS Crosslinked 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

1. Prepare 2M hydroxylamine•HCl by adding it to PBS and adjusting the pH back to 8.5. Prepare this solution immediately before use.
2. 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; however, efficiency might be lower.

   Note: An aliquot of the hydroxylamine cleaved sample, containing 13-14µg quantities of protein, can be examined by SDS-PAGE to determine effectiveness of the cleavage.

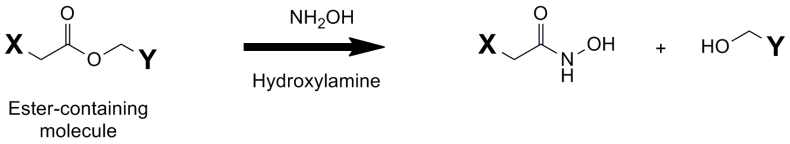


Figure 1. Reaction scheme of EGS cleavage using hydroxylamine•HCl.

Additional Information Available on Our Website

- Tech Tip #43: Protein stability and storage
- Tech Tip # 3: Determine reactivity of NHS ester biotinylation and crosslinking reagents

Related Thermo Scientific Products

25200-44	**Precise™ Protein Gels** (see catalog or website for a complete listing)
24590	**GelCode™ Blue Stain Reagent,** 500mL
23225	**Pierce BCA Protein Assay Kit**

General References

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Baskin, L.S. and Yang, C.S. (1980). Cross-linking studies of cytochrome P-450 and reduced nicotinamide adenine nucleotide phosphate-cytochrome P-450 reductase. *Biochemistry* **19:**2260-4.

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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Conn, P.M., *et al.* (1982). Conversion of a gonadotropin-releasing hormone antagonist to an agonist. *Nature* **296:**633-55.

Conn, P.M., *et al.* (1982). Potency enhancement of a GnRH agonist: GnRH-receptor microaggregation stimulates gonadotropin release. *Endocrinology* **111:**335-7.

Millar, J.B. and Rozengur, E. (1990). Chronic desensitization to bombesin by progressive down-regulation of bombesin receptors in Swiss 3T3. *J Biol Chem* **265:**12052-8.

Partis, M.D., *et al.* (1983). Cross-linking of protein by maleimido alkonyl *N*-hydroxysuccinimido esters. *J Prot Chem* **2(3):**263-77.

Wood, C.L. and O'Dorisio, M.S. (1985). Covalent cross-linking of vasoactive intestinal polypeptide to its receptors on human lymphoblasts. *J Biol Chem* **260:**1243-7.

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