INSTRUCTIONS

# DSG

## 20593

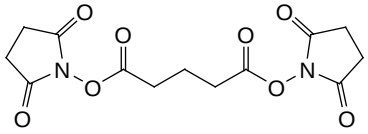
0543.3

| Number | Description |
|---|---|
| **20593** | **DSG** (disuccinimidyl glutarate), 50mg |

Formula: $C_{13}H_{14}N_2O_8$

Spacer arm: 7.7Å

Molecular weight: 326.26



**Storage:** Upon receipt store desiccated at 4°C. Product is shipped at ambient temperature.

## Introduction

Thermo Scientific DSG is a water-insoluble, homobifunctional *N*-hydroxysuccinimide ester (NHS-ester) crosslinker often used for conjugating radiolabeled ligands to cell-surface receptors.[1] The NHS ester is the simplest and most commonly used reactive group for crosslinking and labeling proteins and peptides. NHS esters react with primary amines on the N-termini of peptides and the ε-amine of lysine residues, forming a stable, covalent amide bond and releasing the NHS group.

## Important Product Information

- DSG is moisture-sensitive. To avoid moisture condensation onto the product, vial must be equilibrated to room temperature before opening. For optimal results, store DSG under nitrogen.
- Because NHS-esters hydrolyze readily, prepare DSG immediately before use. Do not store DSG in solution. Solvents commonly used to dissolve insoluble NHS-esters (DMSO or DMF) are hygroscopic and tend to absorb water and promote DSG hydrolysis.
- Hydrolysis of the NHS-ester is a major competing reaction of the NHS-ester acylation reaction. Rate of hydrolysis increases with increasing pH and in dilute protein or peptide solutions. In more concentrated solutions the acylation reaction is favored.
- NHS-ester crosslinking reactions are most often performed in phosphate, carbonate/bicarbonate, HEPES, and borate buffers. Other buffers can also be used provided they do not contain primary amines. Tris and glycine contain primary amines in their structure and are not suitable components for reaction buffers. A large excess of Tris or glycine at neutral-to-basic pH can be added to quench the reaction.
- Crosslinking reactions with water-insoluble NHS-esters are typically performed with a solvent carry-over of up to 10% final volume in the aqueous reaction. These reagents begin to precipitate at high concentrations and appear as a milky, turbid solution. While crosslinking still may occur, the protocol may be modified to ensure complete dissolution of the NHS-ester. For example, the aqueous phase can be supplemented with additional organic solvents.
- Crosslinking proteins that display biological activity (e.g., enzymes, antibodies, etc.) may lose activity upon conjugation. This loss of activity may be a result of a conformational change in the protein or when the crosslinker modifies lysine groups involved in binding substrate (in the case of an enzyme) or an antigen (in the case of an antibody).

## Materials Required

- Crosslinker solution: Dissolve DSG in dry DMSO at 10-25mM
- Reaction buffer: Phosphate-buffered saline (PBS; 0.1M phosphate, 0.15M sodium chloride; pH 7.2; Product No. 28372). Alternative buffers include HEPES, bicarbonate/carbonate or borate buffers at pH 7-9. Do not use buffers containing Tris or glycine.
- 1M Tris, pH 7.5 (for quenching)

## General Procedure for Crosslinking Proteins

1. Prepare the protein sample in reaction buffer.
2. Add DSG to the sample at a final concentration of 0.25-5mM. If the protein is > 5mg/mL, add a 10-fold molar excess of DSG to the protein. If the protein is < 5mg/mL, add a 20- to 50-fold molar excess of DSG.
3. Incubate reaction mixture at room temperature for 30 minutes or on ice for 2 hours.
4. Quench the reaction for 15 minutes with Tris or glycine. Use a final concentration in the reaction mixture of Tris or glycine at 20-50mM.

## General Procedure for Crosslinking Integral Membrane Proteins

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

## Related Thermo Scientific Products

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| **20673** | **Dimethyl formamide (DMF), Sequanal grade,** 50mL |
| **20688** | **Dimethyl sulfoxide (DMSO), Sequanal grade,** 950mL |
| **28372** | **BupH™ Phosphate Buffered Saline Packs,** 40 packs |
| **89889** | **Zeba Spin Desalting Columns, 7K MWCO, 2mL,** 5/pkg |
| **89891** | **Zeba Spin Desalting Columns, 7K MWCO, 5mL,** 5/pkg |
| **20036** | **Bioconjugate Techniques, 2nd Edition,** softcover |

### General Reference

Carlsson, J., *et al.* (1978). Protein thiolation and reversible protein-protein conjugation. *N*-succinimidyl 3-(2-pyridyldithio)propionate, a new heterobifunctional reagent. *Biochem J* **173:**723-37.



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