INSTRUCTIONS

BSOCOES

21600

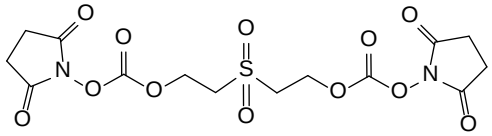
0547.3

Number	Description
21600	**BSOCOES** (bis[2-(succinimidyloxycarbonyloxy)ethyl]sulfone), 50mg

Molecular Weight: 436.36

Spacer Arm Length: 13.0Å

Formula: $C_{14}H_{16}N_2O_{12}S$



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

Introduction

Thermo Scientific BSOCOES is a homobifunctional *N*-hydroxysuccimide ester (NHS ester) crosslinker that effectively forms covalent bonds with molecules having primary amines such as peptides and proteins. The spacer arm in BSOCOES contains a sulfone bond, which can be cleaved with alkaline conditions to reverse the linkage. For example, peptides crosslinked using BSOCOES can be electrophoresed in a reducing gel and then the spacer arm cleaved by treatment of excised gel band with base (pH 11.6) for 2 hours at 37°C.[1] The reagent has been used for conjugating radiolabeled ligands to cell surface receptors.[1-3]

The NHS-ester group reacts with α-amino groups present at the N-termini and ε-amines in the side chain of lysine residues. Although five amino acids have nitrogen in their side chains, only the ε-amines of lysine react significantly with NHS esters. The reaction results in the release of N-hydroxysuccinimide (NHS), which absorbs strongly at 260-280nm (and thereby interferes with assessment of protein concentration by absorbance measurement at 280nm). Hydrolysis of the NHS-ester group significantly competes with the NHS ester acylation reaction. The rate of hydrolysis increases with increasing pH; therefore, reactions with NHS esters are generally performed in buffers at pH 7.2-8.0. NHS ester crosslinking reactions are usually performed in phosphate, carbonate/bicarbonate, HEPES or borate buffers. Other amine-free buffers are also acceptable. A large molar excess of amine-containing reagents (e.g., Tris or glycine) at alkaline pH can be added at the end of a crosslinking reaction for quenching.

Important Product Information

- BSOCOES does not possess a charged group, is lipophilic, and therefore, membrane-permeable and useful for intracellular and intramembrane conjugation. BSOCOES is not water-soluble and must be dissolved in organic solvent (e.g., DMSO or DMF) immediately before use. Stock solutions are not stable because DMSO and DMF are hygroscopic and hydrolysis of the NHS ester will occur.
- Generally, to maintain functionality and solubility of proteins do not exceed 10% DMSO in the final reaction.
- When added to aqueous buffers, BSOCOES in organic solvent might form a microprecipitate resulting in a milky appearance. This will not affect the crosslinking results.
- Add a 10-fold molar excess of the 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. Generally, this range of molar excesses is accomplished with crosslinker at a final concentration of 0.25-5.0mM.

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Additional Materials Required

- Solvent: dry (i.e., free of moisture) dimethyl sulfoxide (DMSO) or dimethyl formamide (DMF)
- Conjugation Buffer: Phosphate-buffered saline (PBS; 20mM sodium phosphate, 0.15M sodium chloride, pH 7.5); alternatively HEPES, bicarbonate/carbonate, borate or other amine-free buffers (pH 7-9) may be used.
- Quenching Solution: 1M Tris•HCl, pH 7.5

Procedure for Crosslinking Amine-containing Molecules in Solution

1. Prepare sample (e.g., protein) in conjugation buffer.
2. Fully equilibrate vial of desiccated BSOCOES to room temperature before opening.
3. Dissolve 2-4mg of BSOCOES with solvent to 10-25mM.
4. Immediately add the dissolved BSOCOES to the protein sample to achieve a concentration equal to 10-50 times the molar concentration of proteins in solution (See Important Product Information).
5. Incubate the reaction mixture at room temperature for 30 minutes or on ice for 2 hours.
6. Add Quenching Solution to a final concentration of 20-50mM and incubate reaction for 15 minutes.
7. If desired, excess non-reacted crosslinker and reaction byproducts can be removed by dialysis or desalting.

Procedure for Inter- and Intracellular Crosslinking of Proteins

Note: BSOCOES is membrane permeable and will crosslink both extracellular and intracellular proteins.

1. Fully equilibrate vial of desiccated BSOCOES to room temperature before opening.
2. Wash cells (adherent or monolayer) in cold PBS or other conjugation buffer to remove amine-containing culture media. If manipulating a specific receptor-ligand interaction, incubate cells or membranes (0.1-0.5mg) with the ligand (5-10nM) in PBS (100µL) for 1 hour at 4°C. Cells can be washed with PBS to remove excess non-bound ligand and then kept in PBS at 4°C.
3. Dissolve 2-4mg of BSOCOES with solvent to 10-25mM.
4. Immediately add the dissolved BSOCOES to the prepared cells to a final concentration of 1-2mM.
5. Incubate the reaction mixture for 0.5-2 hours at room temperature or 4°C, as appropriate for the cells.
6. Add Quenching Solution to a final concentration of 20-50mM and incubate for 15 minutes. Alternatively, cells can be washed with Quenching Solution to remove and quench non-reacted crosslinker and byproducts.

Procedure for Base-Cleavage of BSOCOES

1. Increase the pH of the solution containing the BSOCOES conjugate to 11.6 with NaOH.
2. Incubate for 2 hours at 37°C.
3. Desalt or dialyze the sample into an appropriate buffer for subsequent analysis.

Cited References

1. Zarling D.A., *et al.* (1980). Mapping of lymphocyte surface polypeptide antigens by chemical crosslinking with BSOCOES. *J Immunol* **124:**913-20.
2. Pilch, P.F. and Czech, M.P. (1979). Interaction of crosslinking agents with the insulin effector system of isolated fat cells. *J Biol Chem* **254:**3375-81.
3. Howard, A., *et al*. (1985). Covalent labeling of opioid receptors with human β-endorphin. *J Biol Chem* **260:**10833-9.

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66382	**Slide-A-Lyzer® Dialysis Cassette Kit, 10K MWCO, 3mL**
66807	**Slide-A-Lyzer Dialysis Cassette Kit, 10K MWCO, 12mL**
28372	**BupH™ Phosphate Buffered Saline Packs,** 40 packs
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23235	**Pierce Micro BCA Protein Assay Kit**
23236	**Coomassie Plus™ (Bradford) Assay Kit**
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