INSTRUCTIONS

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Sulfo-SAED

33030

<table>
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<th>Number</th>
<th>Description</th>
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<tr>
<td>33030</td>
<td>Sulfo-SAED, sulfosuccinimidyl 2-(7-azido-4-methylcoumarin-3-acetamido)ethyl-1,3'-dithiopropionate</td>
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Package Size: 5 mg
Molecular Weight: 621.6
Spacer Arm: 23.6 Å
AMCA Properties:
- Excitation maximum: 345 nm
- Emission maximum: 440-460 nm
Extinction Coefficients:
- 18,200 M⁻¹ cm⁻¹ at 327 nm in acetonitrile:water (15:2 v/v), measured using a 1 mg/ml solution
- 13,625 M⁻¹ cm⁻¹ at 298 nm in acetonitrile:water (15:2 v/v), measured using a 1 mg/ml solution
Solubility:
- 3 mg/ml in water
- 2.5 mg/ml in acetone containing a small amount of water
- 50 mg/ml in dimethylsulfoxide (DMSO) or dimethylformamide (DMF)

Storage: Upon receipt store desiccated at 4°C and protected from light.

Introduction

Sulfo-SAED is a water-soluble, heterobifunctional, cleavable crosslinker with a fluorescent spacer arm. The sulfo-NHS ester reacts with primary amines (-NH₂) to form stable amide bonds. The aryl azide group reacts with nucleophiles or any N-H and C-H bond when activated with UV light. In addition to possessing these crosslinking functionalities, Sulfo-SAED is fluorescent as a result of the azidomethylcoumarin acetamide (AMCA) group incorporated into the photoactivatable end of the spacer arm. The spacer arm also includes a disulfide bond that may be cleaved with reducing agent to separate crosslinked molecules, thereby leaving the fluorescent AMCA group attached to the molecule that was conjugated by the photoreaction.

Sulfo-SAED was developed as a means of transferring a fluorescent tag between interacting proteins. Thevenin et al.¹ reacted soybean trypsin inhibitor (STI) in the dark with a 3-fold molar excess of Sulfo-SAED. After removing the nonreacted Sulfo-SAED by gel filtration, an equimolar amount of trypsin was added and reacted to the modified STI by activating the aryl azide group with UV light. Analysis indicated that the resulting STI-trypsin conjugate contained the fluorescent AMCA label. Unconjugated trypsin was not labeled by the fluorophore, while trypsin recovered after reduction of the purified conjugate was labeled with AMCA. When excess unmodified (competitor) STI was included in the reaction, label transfer did not occur. To demonstrate that the successful crosslinking resulted from a specific protein interaction between STI and trypsin and not just random association of proteins in solution, the trypsin was replaced with carbonic anhydrase, which has no affinity for STI. When the experiment was repeated in this way, no crosslinking or label transfer was observed.

Sulfo-SAED was also used as a crosslinker and label-transfer reagent to examine the role of foot protein and Ca²⁺ release from sarcoplasmic reticulum.² Sulfo-SAED was conjugated to polylysine and neomycin (Ca²⁺ inducers), which bind to different portions of foot protein. The modified molecules were then incubated with foot protein and photoactivated. Subsequent reduction of the crosslinker disulfide bond released polylysine and the neomycin, leaving the AMCA label attached to foot protein at sites corresponding to polylysine or neomycin attachment. The experimental design enabled fluorescence detection of calcium-dependent conformational changes in the foot protein.
Considerations for Use of Sulfo-SAED

- Sulfo-SAED is moisture-sensitive. To avoid moisture condensation onto the product, equilibrate the vial to room temperature before opening. Prepare aqueous stock solution immediately before use, and do not attempt to store them as the NHS ester will hydrolyze, making the product non-reactive. Stock solutions prepared in anhydrous DMSO, DMF or acetonitrile may be stored for several days or weeks.

- Hydrolysis of the NHS ester is a competing reaction whose rate increases with increasing pH. Hydrolysis occurs more readily in dilute protein or peptide solutions. In concentrated protein solutions, the desired acylation reaction is favored.

- Use amine-free buffers at pH 7-9 such as 20 mM sodium phosphate, 0.15 M NaCl (Product No. 28372); 20 mM HEPES; 100 mM carbonate/bicarbonate; or 50 mM borate. Do not use buffers that contain Tris, glycine or sulfhydryls. Tris and glycine will compete with the intended reaction, and thiols can reduce the azido group.

- For protein concentration greater than 5 mg/ml, use a 10-fold molar excess of the crosslinker. For samples <5 mg/ml, use a 20- to 50-fold molar excess of the crosslinker. Use a final concentration of crosslinker at 0.1-10 mM.

- The wavelength range required to photoactivate Sulfo-SAED is in the visible part of the light spectrum. Therefore, expensive UV lamps are not required to crosslink with this reagent. Several bright camera flashes are usually sufficient to activate the azido group. Exposure to long wave UV (7 minutes), short wave UV (7 minutes), or to a 250 watt light bulb (1 minute) will all activate the azido group. Visit the Pierce web site for additional information on photoactivation, including the Tech Tip: Light sources and conditions for photoactivation of aryl azide cross-linking reagents.

- To cleave the disulfide bond between crosslinked proteins, use 10-50 mM DTT at 37°C for 30 minutes or 5% 2-ME in SDS-PAGE sample buffer (2% SDS, 6.25 mM Tris base, 10% glycerol) at 100°C for 5 minutes.

- For best results in label-transfer protein interaction experiments, use the following experimental strategy:
  1. Label purified Protein#1 in the dark in amine-free buffer for 1 hour using a molar fold excess of Sulfo-SAED.
  2. Desalt labeled Protein#1 in the dark. (e.g., using Zeba™ Desalt Spin Columns, Product No. 89889 or 89891).
  3. Add labeled Protein#1 in the dark to solution containing Protein#2 and allow the interaction to occur.
  4. Photoactivate the aryl azide group to complete the crosslinking reaction.
  5. Purify and/or analyze the conjugate, or cleave the disulfide spacer arm and then characterize the protein interactors by detecting AMCA fluorescence.

Related Pierce Products

33033  Sulfo-SBED, amine and photoactivatable biotin transfer reagent
33093  Mts-Atf-Biotin Label Transfer Reagent, sulfhydryl and photoactivatable biotin transfer reagent

Cited References


Product References


Current versions of product instructions are available at www.piercenet.com. For a faxed copy, call 800-874-3723 or contact your local distributor.

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