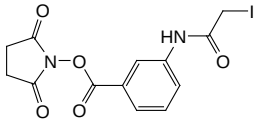
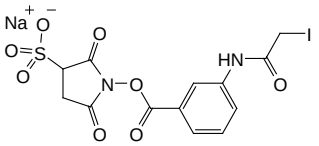
INSTRUCTIONS

SIAB
Sulfo-SIAB

22329 22327

0439.2

Number	Description
22329	**SIAB** (*N*-succinimidyl[4-iodoacetyl]aminobenzoate), 50mg Molecular Weight: 402.15 Spacer Arm: 10.6Å  **Storage:** Upon receipt store SIAB protected from light and desiccated at 4°C. Reagent is shipped at ambient temperature.
22327	**Sulfo-SIAB** (sulfosuccinimidyl[4-iodoacetyl]aminobenzoate), 50mg Molecular Weight: 504.20 Spacer Arm: 10.6Å  **Storage:** Upon receipt store Sulfo-SIAB protected from light and desiccated at -20°C. Reagent is shipped at ambient temperature.

Introduction

Thermo Scientific SIAB is an amine- and sulfhydryl-reactive heterobifunctional crosslinker and Sulfo-SIAB is its water-soluble analog. These crosslinkers contain an amine-reactive *N*-hydroxysuccimide (NHS) ester and a sulfhyryl-reactive iodoacetyl group, and are often used for preparing enzyme conjugates or immunotoxins. Water-soluble and water-insoluble forms of NHS esters have essentially identical reactivity. Sulfo-SIAB is supplied as a sodium salt and is water-soluble to ~10mM. SIAB must be first dissolved in an organic solvent, such as DMSO or DMF, and then added to the aqueous reaction mixture. SIAB is lipophilic, membrane-permeable and does not possess a charged group.

NHS esters react with primary amino groups (-NH_2) present on the side chain of lysine (K) residues and the N-terminus polypeptides. The reaction proceeds efficiently in pH 7-9 buffers to form stable amide bonds and results in the release of *N*-hydroxysuccinimide. Hydrolysis of the NHS ester is a competing reaction and increases with increasing pH. Hydrolysis occurs readily in dilute protein solutions; the acylation reaction is favored in concentrated protein solutions.

Iodoacetyl groups react with the free sulfhydryls by nucleophilic substitution of iodine with a thiol group resulting in a stable thioether linkage. Sulfhydryl specificity occurs by using a slight stoichiometric excess of iodoacetyl groups over the number of free sulfhydryls present and by maintaining the reaction at pH 7.5-8.5. The reaction is most specific for sulfhydryl groups at pH 8.3. If there are no free sulfhydryls present, or if there is a gross excess of iodoacetyl group over sulfhydryls, the iodoacetyl group can react with other amino acids. Imidazoles also can react with iodoacetyl groups at pH 6.9-7.0, but the incubation must proceed for at least a week. Histidyl side chains and amino groups react in the unprotonated form with iodoacetyl groups above pH 5 and pH 7, respectively.

Important Product Information

- These crosslinkers are moisture-sensitive. 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, do not prepare stock solutions for storage. Discard any unused reconstituted crosslinker.

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- Use a non-amine-containing reaction buffer at pH 7-9 such as 20mM sodium phosphate, 0.15M sodium chloride (Product No. 28372); 20mM HEPES; 100mM carbonate/bicarbonate; or 50mM borate (Product No. 28384). Avoid using Tris or glycine as buffer components, as they will compete with the intended reaction.
- Exclude reducing agents, such as 2-mercaptoethanol, dithiothreitol, and mercaptoethylamine from reaction buffers, as these compounds will quench the iodoacetyl reactivity. Also, prepare solutions and perform reactions in the dark to limit generation of free iodine, which can potentially react with tyrosine, histidine, and tryptophan residues.
- Some sulfhydryl-containing peptides and proteins may oxidize in solution and form disulfide bonds, which cannot react with iodoacetyl groups. Disulfide bonds can be reduced to produce free sulfhydryls. Themo Scientific Immobilized Reductant Columns (Product No. 77701) and Immobilized TCEP Disulfide Reducing Gel (Product No. 77712) enable peptide or protein reduction while recovering the sample in the absence of reducing agents.
- Sulfhydryls can be introduced via amine modification using *N*-succinimidyl *S*-acetylthioacetate (SATA, Product No. 26102) or 2-iminothiolane•HCl (Traut's Reagent, Product No. 26101).

Protocol for Preparing IgG/β-Galactosidase Conjugates

The following protocol is a two-step method in which an iodoacetyl-activated IgG is prepared in the first step. The activated IgG is then reacted with free sulfhydryls present on the surface of native β-galactosidase. Modify this method to optimize the ratio of IgG to β-galactosidase.

Materials

- Borate buffer: 50mM sodium borate, pH 8.5 (Product No. 28384), 5mM EDTA
- 1mg/mL IgG in borate buffer
- Thermo Scientific Zeba Spin Desalting Columns, 10mL (Product No. 89894), or other device to remove nonreacted reagents
- Cysteine•HCl (Product No. 44889)

Method

1. Just before use, dissolve 1.4mg SIAB in 1mL DMSO or dissolve 1.7mg Sulfo-SIAB in 1mL of ultrapure water. Protect solutions from light.
2. Add 10µL of crosslinker solution to 1mL of IgG and react for 30 minutes at room temperature.
3. Remove nonreacted crosslinker using a desalting column equilibrated with borate buffer.
4. Add 4mg of β-galactosidase to the desalted IgG and react for 1 hour at room temperature in the dark.
5. To quench the reaction, add a final concentration of 5mM cysteine and react for 15 minutes at room temperature in the dark.
6. Remove nonreacted reagents by desalting or dialysis.

Related Thermo Scientific Products

23235 **Micro BCA Protein Assay Kit**

20036 **Bioconjugate Techniques,** 2nd Edition

General Reference

Weltman, J.K., *et al.* (1983). *N*-Succinimidyl (4-iodoacetyl) aminobenzoate: a new heterobifunctional crosslinker. *Biotechniques* **1:**148-52.

This product ("Product") is warranted to operate or perform substantially in conformance with published Product specifications in effect at the time of sale, as set forth in the Product documentation, specifications and/or accompanying package inserts ("Documentation") and to be free from defects in material and workmanship. Unless otherwise expressly authorized in writing, Products are supplied for research use only. No claim of suitability for use in applications regulated by FDA is made. The warranty provided herein is valid only when used by properly trained individuals. Unless otherwise stated in the Documentation, this warranty is limited to one year from date of shipment when the Product is subjected to normal, proper and intended usage. This warranty does not extend to anyone other than the original purchaser of the Product ("Buyer").

No other warranties, express or implied, are granted, including without limitation, implied warranties of merchantability, fitness for any particular purpose, or non infringement. Buyer's exclusive remedy for non-conforming Products during the warranty period is limited to replacement of or refund for the non-conforming Product(s).

There is no obligation to replace Products as the result of (i) accident, disaster or event of force majeure, (ii) misuse, fault or negligence of or by Buyer, (iii) use of the Products in a manner for which they were not designed, or (iv) improper storage and handling of the Products.

Current product instructions are available at *www.thermoscientific.com/pierce*. For a faxed copy, call 800-874-3723 or contact your local distributor.

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