INSTRUCTIONS

SATA and SATP

26102 26100

0126.3

Number	Description
26102	**SATA (*N*-Succinimidyl S-Acetylthioacetate),** 50 mg Molecular Weight: 231.23 Spacer Arm Length (from amine to sulfur after conjugation): 2.8 Å CAS #76931-93-6
26100	**SATP (*N*-Succinimidyl S-Acetylthiopropionate)**, 50 mg Molecular Weight: 245.25 Spacer Arm Length (from amine to sulfur after conjugation): 4.1 Å CAS #84271-78-3 **Storage**: Upon receipt store desiccated at -20°C. Product is shipped at ambient temperature.

Introduction

SATA and SATP are reagents for introducing protected sulfhydryls into proteins, peptides and other molecules. They are the *N*-hydroxysuccinimide (NHS) esters of S-acetylthioacetic and propionic acid (Figure 1). A stable, covalent amide bond is formed from the reaction of the NHS ester with primary amines. The amine reacts with the NHS ester by nucleophilic attack, with *N*-hydroxysuccinimide being released as a by-product. Deprotection (deacylation) to generate a sulfhydryl for use in cross-linking and other applications is accomplished using hydroxylamine•HCl (See Related Thermo Scientific Products).

Sulfhydryl groups present on proteins, peptides and other compounds are important in protein chemistry/modification reactions. In several cases, thiols are unavailable or absent within the molecules of interest. While several reagents and techniques[1,2] are available for introducing sulfhydryl groups or disulfides into proteins and peptides including Traut's Reagent and SPDP (see Related Thermo Scientific Products at the end of these instructions), SATA and SATP have several features and benefits for sulfhydryl addition applications:

- Reaction conditions are mild and non-denaturing. NHS ester reactions may be performed in a variety of non-amine buffers at pH 7-9 and temperatures 4-37°C, with incubation times ranging from few minutes to overnight.
- The reaction is specific toward primary amines. See Appendix for a schematic of the SATA reaction.
- Sulfhydryl groups are introduced in a protected form, allowing the modified molecule to be stored indefinitely and then later treated with hydroxylamine to expose the labile sulfhydryl group for final conjugation reactions.

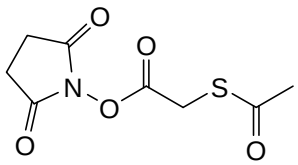

Figure 1a. Molecular structure of SATA (*N*-succinimidyl S-acetylthioacetate).

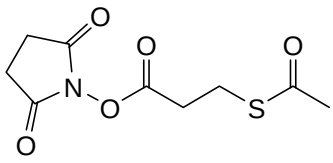

Figure 1b. Molecular structure of SATP (*N*-succinimidyl S-acetylthiopropionate).

Pierce Biotechnology 3747 N. Meridian Road | PO Box 117 Rockford, IL 61105 USA | (815) 968-0747 (815) 968-7316 fax | www.thermo.com/pierce

Important Product Information

- SATA and SATP are moisture-sensitive. Store desiccated at -20°C. To avoid moisture condensation in the product, fully equilibrate the vial to room temperature before opening.
- Dissolve reagent immediately before use. The NHS-ester moiety readily hydrolyzes, making the reagent nonreactive; therefore, reagent solutions must not be stored as stocks. Discard any unused reagent solution.
- Avoid using buffers that contain amines (e.g., Tris and glycine) because amines directly compete with the reaction. Phosphate Buffered Saline (PBS) or HEPES buffers at pH 7.2-8.0 are good options for applications involving proteins.
- Both the acylation reaction to primary amines and the hydrolysis (inactivation) of these NHS-ester reagents occurs more rapidly at higher pH. For this reason, procedures for modification with SATA and SATP involve addition of a molar excess of reagent, and reactions proceed to completion (modification or hydrolysis) in minutes (pH 9) or hours (pH 7).
- As the target amines are more concentrated, the intended acylation reaction is more favored over hydrolysis.

Additional Materials Required

- D-Salt™ Excellulose™ Desalting Column, 5 ml (Product No. 20449)
- Hydroxylamine•HCl (Product No. 26103)
- Phosphate Buffered Saline (PBS, Product No. 28372)
- EDTA and 1 N NaOH for modifying PBS buffer
- DMSO (Dimethylsulfoxide, Product No. 20688)

Material Preparation

Reaction Buffer	Prepare 200-500 ml of PBS: 0.1 M phosphate, 0.15 M NaCl, pH 7.2-7.5
Protein Solution	Dissolve protein to be modified in Reaction Buffer to a concentration of 60 µM (2-10 mg/ml). For an IgG with 150,000 molecular weight, 60 µM corresponds to 9 mg/ml.
Deacetylation Solution	0.5 M Hydroxylamine, 25 mM EDTA in PBS, pH 7.2-7.5. Dissolve 1.74 g hydroxylamine•HCl and EDTA (0.475 g of tetrasodium salt or 0.365 g of disodium salt) in 40 ml of Reaction Buffer. Add ultrapure water to a final volume of 50 ml and adjust pH to 7.2-7.5 with NaOH.

Procedure for Sulfhydryl Modification of Protein

Note: This protocol was used with human IgG to yield incorporation of 3.0-3.6 moles sulhydryl per mole of IgG.

A. Reaction of Antibody with SATA (or SATP)

1. Immediately before reaction, dissolve 6-8 mg of SATA (or SATP) in 0.5 ml of DMSO (results in ~55 mM solution).
2. Combine 1.0 ml of Protein Solution with 10 µl of the SATA solution. Mix contents and incubate reaction at room temperature for 30 minutes.

   Note: The level of sulfhydryl incorporation may be altered by using different molar ratios of SATA to protein. This default reaction uses 60 nmol Protein and 550 nmol SATA, a 9:1 molar ratio of SATA to protein. More complete acylation of all primary amino groups will occur when larger molar excesses of SATA are used (Table 1); however, higher levels of acylation correspond to greater risk of protein inactivation. Increase or decrease the amount of SATA in the reaction by adding more or less than 10 µl of the SATA solution per ml of Protein Solution.

Table 1. Effect of varying molar ratios of SATA on sulfhydryl incorporation onto bovine serum albumin (BSA, MW 67,000). Sulfhydryl incorporation increased with greater amounts of SATA, but with decreasing efficiency.

Molar Ratio of SATA to Protein	Moles of sulhydryls incorporated per mole BSA
25:1	21.16
50:1	23.60
100:1	29.37
250:1	32.65

B. Desalt to Purify Acylated Protein from Excess Reagent and By-Products

Note: Dialysis may be performed as an alternative to using a desalting column for this section of the procedure.

1. Equilibrate a desalting column with two column volumes of Reaction Buffer. Use at least a 5 ml desalting column for each 1 ml of reaction volume to be processed.
2. Apply the 1.01 ml reaction mixture to column. Immediately begin collecting 1 ml fractions. When the reaction mixture has completely entered the column bed and the first fraction collected, add Reaction Buffer to the column and continue collecting separate 1 ml fractions as they emerge from the column.
3. Identify fraction(s) that contain protein by measuring for those having peak absorbance at 280 nm. With a 5 ml desalting column, fractions 2 and 3 will contain most of the protein. Pool fractions that contain the protein.

   Note: At this point, the modified protein may be stored indefinitely for later deacetylation and generation of sulfhydryl groups (Section C).

C. Deacetylate SATA-Modified Protein to Generate Sulfhydryl Groups

1. Combine 1.0 ml of SATA-modified (acetylated) protein with 100 µl of the Deacetylation Solution.
2. Mix contents and incubate reaction for 2 hours at room temperature.
3. Use a desalting column to purify the sulfhydryl-modified protein from the Hydroxylamine in the Deacetylation Solution. Desalt into Reaction Buffer containing 10 mM EDTA to minimize disulfide bond formation using the same procedure as in Section B. Promptly use the prepared protein in the end application. Before or after desalting, the protein may be assayed for sulfhydryl content using Ellman's Reagent (see Related Thermo Scientific Products).

Related Thermo Scientific Products

28372	**BupH™ Phosphate Buffered Saline Packs**, 40 packs, each yielding 500 ml of 0.1 phosphate, 0.15 M NaCl, pH 7.2 when dissolved in 500 ml ultrapure water
20688	**DMSO (Dimethylsulfoxide)**, 950 ml
20449	**D-Salt™ Excellulose™ Desalting Columns**, 5 x 5 ml
26103	**Hydroxylamine•HCl**, 25 g
26101	**Traut's Reagent (2-Iminothiolane•HCl)**, 500 mg
21857	**SPDP**, 50 mg
22582	**Ellman's Reagent**, 5 g
23460	**Protein Coupling Handle Addition Kit**, convenient kit for SATA modification and sulfhydryl group detection with Ellman's reagent

Appendix

The reaction scheme for sulfhydryl modification of protein amino groups is best presented in two steps (Figure 2 and 3). In the first step, primary amines on the protein react to form an amide bond with the reagent, which contains a protected sulfhydryl. In the second step, hydroxylamine is used to deacetylate the sulfur, resulting in a sulfhydryl group.

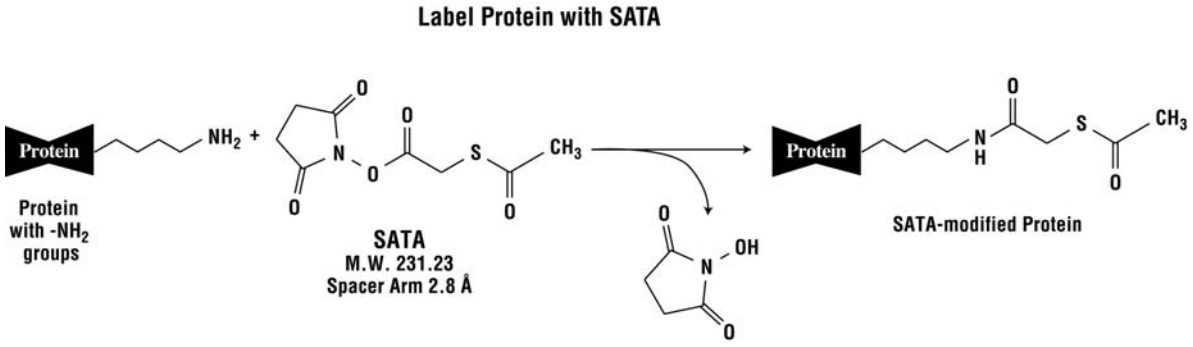

Figure 2. Step 1 is the reaction of SATA with a primary amine.

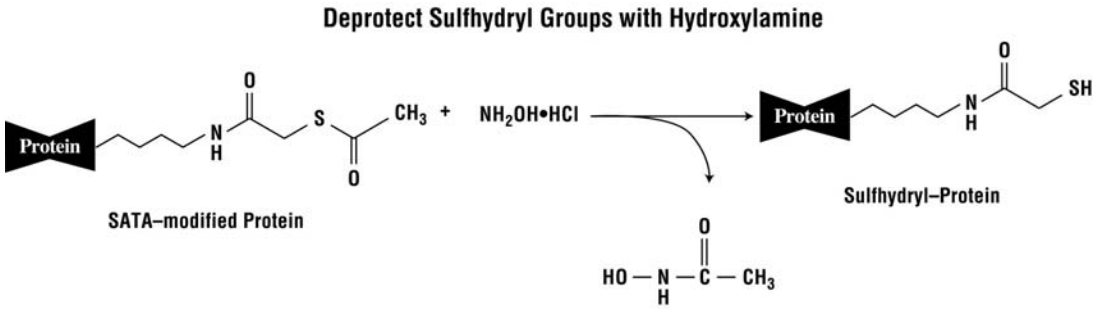

Figure 3. Step 2 is the deprotection with hydroxylamine to generate a sulfhydryl.

Cited References

1. Weston, P.D., *et al.* (1980). Conjugation of enzymes to immunoglobulins using dimaleimides. *Biochem. Biophys. Acta.* **612:**40-9.
2. King, T.P. and Kochoumian, L. (1979). A comparison of different enzyme-antibody conjugates for enzyme-linked immunosorbent assay. *J. Immunol. Methods.* **28**:201-10.

General References

Kumar, A. and Malhotra, S. (1992). A simple method for introducing –SH group at 5' OH terminus of oligonucleotide. *Nucleosides & Nucleotides*. **11(5):** 1003-7.

Duncan, R.J.S., *et al.* (1983). A new reagent which may be used to introduce sulfhydryl groups into proteins, and its use in the preparation of conjugates for immunoassay. *Anal. Biochem.* **132:**68-73.

Product References

Linderfer, M., *et al.* (2001). Targeting of Pseudomonas aruginosa in the bloodstream with bispecific monoclonal antibodies. *J. Immunol.* **167:**2240-9.

Sakharov, D.V., *et al.* (2001). Polylysine as a vehicle for extracellular matrix-targeted local drug delivery, providing high accumulation and long-term retention within the vascular wall. *Aterioscler Thromb Vasc Biol.* **21:**943-948.

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