Thermo SCIENTIFIC

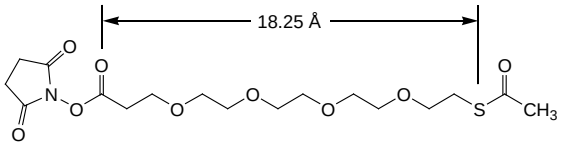
SAT(PEG)$_4$

Amine-reactive thiolating agent with a polyethylene glycol (PEG) spacer arm

26099

1770.3

Number	Description
26099	**SAT(PEG)$_4$,** 100mg Form: Viscous liquid Molecular Weight: 421.46 Spacer Arm: 18.2Å



NHS-PEG$_4$-S-Acetyl
N-succinimidyl S-acetyl (thiotetraethyleneglycol) ester

Storage: Upon receipt store desiccated at -20°C. Product shipped at ambient temperature.

Introduction

Thermo Scientific™ SAT(PEG)$_4$ is a reagent, like SATA and SATP (see Related Thermo Scientific Products), for adding protected sulfhydryl groups to proteins, peptides and other molecules. These reagents contain an *N*-hydroxysuccinimide (NHS) ester, which reacts with primary amine groups, resulting in an irreversible amide bond. The derivative has a stable thioacetate group, which can later be deacetylated with hydroxylamine•HCl to expose the sulfhydryl for crosslinking and immobilization applications (Figure 1).

Although it is like SATA and SATP in general reaction scheme, SAT(PEG)$_4$ is unique in having a long-chain, water-soluble (hydrophilic) polyethylene glycol (PEG) spacer arm. These properties are transferred to the labeled molecule, helping to maintain or add to its solubility. For example, antibodies modified with PEG reagents exhibit less aggregation when stored in solution compared to those modified with purely hydrocarbon groups.

Sulfhydryl groups are useful targets for protein crosslinking applications, and SATA-based reagents provide a means to add sulfhydryl groups when they unavailable or absent within the molecules of interest. SATA-based reagents have several features and benefits for sulfhydryl addition:

- 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 and efficient to primary amines (-NH_2), which occur in proteins at the N-terminus and the side chain of lysine (K) residues.
- Sulfhydryl groups are introduced in a protected form, allowing the modified molecule to be stored indefinitely and then later treated with hydroxylamine•HCl to expose the labile sulfhydryl group for final conjugation reactions.
- By contrast to typical PEG reagents that contain heterogeneous mixtures of different PEG chain lengths, SAT(PEG)$_4$ and other Pierce PEG reagents are homogeneous compounds of defined molecular weight and spacer arm length, providing greater precision in optimization and characterization of crosslinking applications.

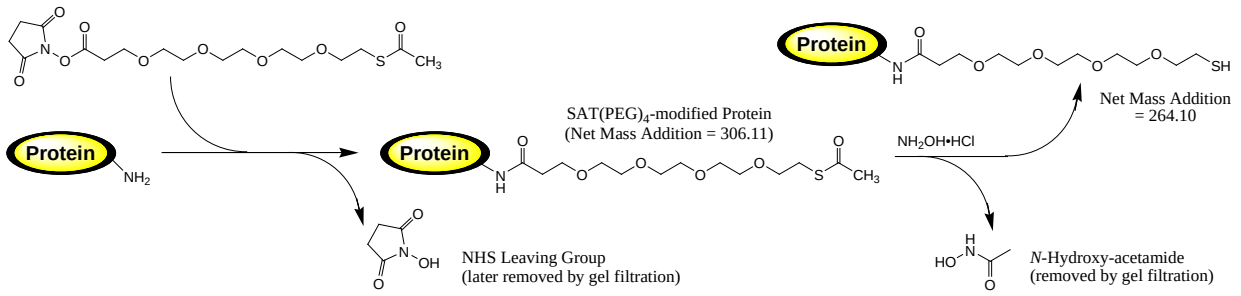


Figure 1. Reaction scheme for the modification of proteins with SAT(PEG)$_4$.

Important Product Information

- $SAT(PEG)_4$ is a viscous pale liquid that is difficult to weigh and dispense. To facilitate handling, make a stock solution immediately before first use by dissolving the crosslinker in dry (anhydrous, molecular sieve-treated) organic solvent, such as dimethylsulfoxide (DMSO, Product No. 20684). Minimize reagent exposure to moisture, as the NHS-ester group is susceptible to hydrolysis. Store unused stock solution in a moisture-free condition (e.g., capped under an inert gas such as argon or nitrogen) at -20°C. Equilibrate reagent vial to room temperature before opening to avoid moisture condensation inside the container. Minimize exposure to air by keeping the stock solution capped by a septum through which reagent can be obtained with a syringe. With proper handling, the stock solution is stable for three months.
- Avoid buffers containing primary amines (e.g., Tris or glycine) during conjugation because they compete with the reaction. If necessary, dialyze or desalt samples into an appropriate buffer such as phosphate-buffered saline (PBS).
- The reagent-to-protein molar ratio affects modification extent of available amine groups. Empirical testing of reagent and protein concentration is necessary to determine optimal conditions for the intended application.

Procedure for Sulfhydryl Modification of Protein

Generally, a 10- to 50-fold molar excess of NHS-ester reagent over the amount of amine-containing protein results in sufficient modification levels for most conjugation applications. Be aware that excessive modification of proteins (e.g., antibodies) can inactivate them by blocking important binding sites. Empirical testing of reagent and protein concentration is necessary to determine optimal conditions for the intended application.

A. Additional Materials Required

- Hydroxylamine•HCl (Product No. 26103)
- DMSO (Dimethylsulfoxide, Product No. 20684)
- Desalting columns (e.g., Thermo Scientific™ Zeba™ Spin Desalting Columns) or dialysis units (e.g., Thermo Scientific™ Slide-A-Lyzer™ Dialysis Cassettes) to separate crosslinked proteins from excess crosslinker and reaction byproducts (see Related Thermo Scientific Products)

B. Material Preparation

Conjugation Buffer	Phosphate-buffered saline (PBS, pH 7.2; e.g., Product No. 28372) or other amine-free buffer at pH 7-8 (see Important Product Information).
$SAT(PEG)_4$ Stock Solution	Read the Important Product Information (previous section) before preparing this solution. Prepare a 250mM $SAT(PEG)_4$ Stock Solution by dissolving 100mg of $SAT(PEG)_4$ (entire contents of vial, approximately 100µL) in 850µL of dry DMSO. Cap, store and handle the stock solution as directed in the previous section.
Quenching Buffer (Optional)	1M Tris•HCl, pH 7.5 (Alternatively, 1M glycine or lysine may be used.)
Deacetylation Solution	0.5M Hydroxylamine, 25mM EDTA in PBS, pH 7.2-7.5. Dissolve 1.74 g hydroxylamine•HCl and EDTA (0.475g of tetrasodium salt or 0.365g of disodium salt) in 40mL of Conjugation Buffer. Add ultrapure water to a final volume of 50mL and adjust pH to 7.2-7.5 with NaOH.

C. Derivatize Protein with $SAT(PEG)_4$

1. Dissolve protein in Conjugation Buffer at 0.1mM (e.g., 5mg in 1mL for a 50kDa protein).
2. Add $SAT(PEG)_4$ to the dissolved protein at 1mM final concentration (= 10-fold molar excess for 0.1mM protein solution) by adding 4µL of $SAT(PEG)_4$ Stock Solution per milliliter of protein solution.
3. Incubate the reaction mixture for 30 minutes at room temperature or 2 hours at 4° C.
4. Quench reaction by adding Quenching Buffer at 20-50mM final and incubating for 15 minutes at room temperature.
5. Remove excess nonreacted reagent and reaction byproducts by gel filtration (desalting column) or dialysis.
6. Proceed immediately to the next section of procedure or store the modified protein (using conditions appropriate for the protein) for later deacetylation and generation of sulfhydryl groups.

D. Deacetylate SAT(PEG)$_4$-Modified Protein to Generate Sulfhydryl Groups

1. Combine 1.0mL of SAT(PEG)$_4$-modified (acetylated) protein with 100µL 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 Conjugation Buffer containing 10mM EDTA to minimize disulfide bond formation. Promptly use the prepared protein in the end application. Before or after desalting, the protein can be assayed for sulfhydryl content using Ellman's Reagent (Product No. 22582).

Related Thermo Scientific Products

89889	**Zeba Spin Desalting Columns, 2mL**
89891	**Zeba Spin Desalting Columns, 5mL**
66385	**Slide-A-Lyzer Dialysis Cassette Kit**
66382	**Slide-A-Lyzer Dialysis Cassette Kit**
26102	**SATA,** 50mg, short-chain amine-reactive thiolating reagent with acetyl protecting group
26100	**SATP,** 50mg, long-chain amine-reactive thiolating reagent with acetyl protecting group
26101	**Traut's Reagent (2-Iminothiolane•HCl)**, 500mg, amine-reactive thiolating reagent without protective group
21857	**SPDP**, 50mg, amine-reactive thiolating reagent with replaceable or reducible pyridyldithiol group
23460	**Sulfhydryl Addition Kit**, complete kit for SATA and Ellman's Reagent

General References

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.

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.

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.

Weston, P.D., *et al.* (1980). Conjugation of enzymes to immunoglobulins using dimaleimides. *Biochem Biophys Acta* **612:**40-9.

Product References (for SATA-type reagents)

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. *Ateriosclerr Thromb Vasc Biol* **21:**943-8.