

FT-84235A

SATA, SATP, SAT-PEO₄-Ac

Products Description

Catalog nb: UP84235B, 50mg UP84235A, 100mg

UP84235C, 250mg

Name: SATA

N-succinimidyl S-acetylthioacetate hydrochloride

Formula: MW = 231.2; CAS [76931-93-61]; (L)

rochloride N-0-C-CH₂SCCH₃

Catalog nb: UPM1175B, 100mg

Name: SATP

N-succinimidyl S-acetylthiopropionate

MW= 245.26; CAS [84271-78-3]; _(L)
Analog of SATA with a 4.1A spacer

Catalog nb: BV2831, 100mg Name: SAT-PEO₄-Ac

N-Succinimidyl S-acetyl(thiotetraethylene glycol)

Formula : MW = 421.46; (L)

Analog of SATA with a 18.25A spacer and PEO

18.25 Å O CH₃

PEO structure confers hydrophilicity to the spacer

Store at $+4^{\circ}$ C, or at -20° C for long term (M)

SATA is a popular agent to convert amine to protected sulfhydryls. It also can be used as a crosslinking agent by thiolating biomolecule then exchanging sulfhydryl with an other molecule [Duncan 1983] SATP is an analog of SATA, with a longer spacer (4.1A).

SAT-PEO₄-Ac, with a longer spacer, also confers hydrophilicity through its long spacer to the conjugate (starting with increased stability and reduced immunogenicity)

Thioacetyl group can be deprotected with 0.02 M Hydroxylamine #05965A to restore a free sulfhydryl [1,2]

Applications

- Conversion of amino group into a sulhydryl for oriented conjugations (NHS/Maleimide method) i.e. SATA used to add thiol groups to rabbit polyclonal IgG [Hermanson 1996] and to Int 8 [Johnson 2004]
- Prevent disulfide formation (ex.peptides)
- Blocage of amino groups in biomolecules (enzymes, receptors)

Technical information

- Thiols are important in protein biochemistry. They are present on several proteins, peptides, but when absent, buried or in insufficient number, they are introduced easily with SATA or iminothiolane (#UP42425)
- NHS group reacts in mild conditions specifically with primary amines, forming a stable covalent linkage, to add protected sulfhydryls. Avoid to use amine containing buffers (Tris, Glycine)! The yield of introduced SH increased with SATA to protein ratio, i.e. 21 SH per BSA at 25: 1 incubation ratio, up 33 SH per BSA with 250: 1 ratio. A calibration should be performed for each protein.
- The by-product of the reaction, N-hydroxysuccinimide, is released and can be monitored by UV.
- When a free sulfhydryl is needed, an easy deprotection step is performed with hydroxylamine (#05965), generating a thioacetylated peptide
- Free sulfhydryl containing peptide can then be conjugated to form a hapten-carrier conjugate



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Directions for Use

Protocol 1: introducing SH in proteins

- 1-Prepare extemporaneously a 15mg/ml solution in dry DMSO
- 2-Prepare 10mg of protein in PBS 1mM EDTA pH7.5
- 3-Add 10µl of SATA to 1ml of protein and incubate for 1H at room temperature
- 4-Desalt the protein by dialysis, ultrafiltration of with desalting columns (UP84874)
- 5-The protein can be stored frozen for long term.

6-When deprotection is needed, Thioacetyl group can be deprotected to restore a free sulfhydryl [Duncan 1983,2]

- -incubate for 2hours at room temperature the protein with 10% v/v of 50 mM sodium phosphate, 25 mM EDTA, 0.5 M hydroxylamine•HCl, pH 7.5.
- -SH content can be assayed with DTNB reagent (#UP01566).
- -Desalting by suitable mean may be required for further use.

References:

Duncan, R.J.S., Weston, P.D. and Wrigglesworth, R. (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. 28:201-210.

Kumar, A. and Malhotra, S. (1992). A simple method for introducing –SH group at 5' OH terminus of oligonucleotide. Nucleosides & Nucleotides. 11(5): 1003-1007.

Weston, P.D., Devries, J.A. and Wrigglesworth, R. (1980). Conjugation of enzymes to immunoglobulins using dimaleimides. Biochem. Biophys. Acta. 612:40-49.

Related / associated products and documents

Hydroxylamine #05965A

*Other amino group modifyers:

- •2-Iminothiolane (Traut's reagent) #UP42425A to convert amino group to un-protected sulfhydryl groups
- •SulfoSuccinimidyl-Acetate (SulfoNHS-Acetate) #UP69380A to block amino groups
- 6-(N-trifluoroacetyl)caproic acid NHS (TFCS) #L7727B to protect amino group that can then be unmasked at pH 7.8-8.1
- •Succinimidyl-p-formyl-benzoate (SFB) #M11771 to convert amino group in CHO groups
- Labeling amino groups: for conversion of amine in biotin tether (labeling), i.e. NHS-Biotins i.e. NHS-PEO4-Biotin UPR2027A, or to fluorescent moiety, i.e. NHS-FluoProbes

*Sulfhydryls modifyers:

•Pyridine dithioethylamine hydrochloride (PDA) #BI1321 to converts sulfhydryl group in amino group.

*Crosslinkers:

*Desalting tools:

CelluSep dialysis tubings

Desalting gelfiltration columns #UP84874

See BioSciences Innovations catalogue and e-search tool.

For in vitro R&D use only

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Rev.IK06E-09E-D12E

