Sulfhydration Kit With SATA

For introducing protected sulfhydryls into proteins

SATA [N-SUCCINIMIDYL S-ACETYLTHIOACETATE]
PROTEIN MODIFICATION KIT

(Cat. # 786-1645)

INTRODUCTION

Sulfhydration Kit With SATA is designed to introduce sulfhydryls into proteins, peptides, antibodies and other molecules which are used in conjugation and labeling of proteins.

SATA [N-Succinimidyly S-Acetylthioacetate] reagent in the kit introduces sulfhydryl groups in the proteins by reacting with primary amines. SATA is N-hydroxysuccinimide (NHS) ester of S-acetylthioacetic and propionic acid (Fig.1). A stable covalent amide bond is formed when SATA reacts with primary amines in the protein. Deprotection or deacylation to generate a sulfhydryl for use in cross-linking is achieved by using hydroxylamine.HCl (Fig.2).

Sulfhydration Kit With SATA contains reagents sufficient for 10 modifications 1 ml of 2-10 mg/ml antibody solutions.

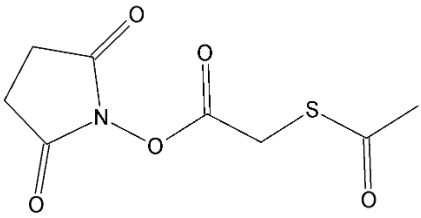


Fig.1: SATA

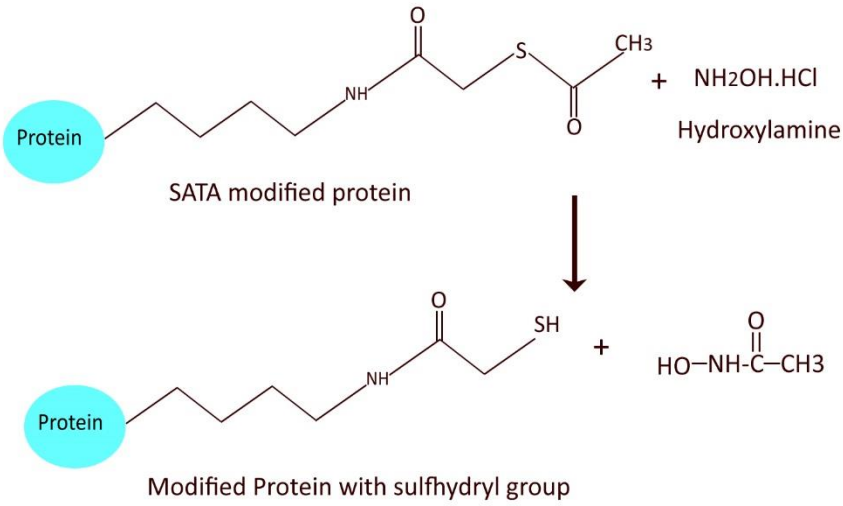


Fig 2: Deacylation with hydroxylamine to generate free SH group

ITEM(S) SUPPLIED

Description	Cat. # 786-1645
SATA	50 mg
DMF	2 x 2ml
Optimizer Buffer™ III [5X]	2 x 25 ml
Hydroxylamine	25 mg
SpinOUT™ GT-600, 5 ml	2 x 5 columns

STORAGE CONDITION

The kit is shipped at room temperature. Upon arrival store the SATA at -20°C and rest of the components at 4°C.

ADDITIONAL REAGENTS REQUIRED

- Protein or antibody solution (2-10 mg/ml) to be modified in amine free neutral pH buffer.

IMPORTANT INFORMATION

- The level of sufhydryl incorporation may be altered by using different 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. Therefore, optimize the SATA to protein ratio before use.

Molar ratio of SATA to BSA	Moles of sulfhydryl incorporated per mole of BSA
25:1	21.16
50:1	23.60
100:1	29.37

Table1: Effect of varying ratio of SATA on sulfhydryl incorporation of BSA (MW: 67,000g/mol)

PREPARATION BEFORE USE

Dilute Optimizer Buffer™ III [5X] with deionized water in ratio 1:4 (e.g. 1 ml of Optimizer Buffer™ III [5X] and 4 ml of deionized water) to get 1X Optimizer Buffer™ III solution.

PROTOCOL

Reaction of SATA with antibody solution

1. Dissolve 4 mg of SATA in 250 µl of DMF immediately before use.
2. Add 10 µl of SATA solution to 1 ml of (2-10 mg/ml) antibody or protein solution.

3. Mix contents and incubate reaction at room temperature for 30 minutes.
4. The modified antibody or protein solution is stable and can be stored at -20°C.

Deacetylation of SATA modified protein for generation of sulfhydryl groups for coupling reactions

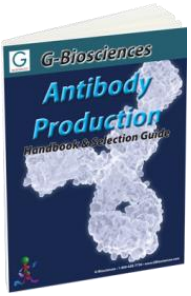
1. Weigh 2 mg hydroxylamine in a clean tube and add 100 µl 1 X Optimizer Buffer™ III. Dissolve to prepare deacetylation solution.
   NOTE: *Prepare immediately before use.*
2. Add 50 µl of deacetylation solution to 1ml SATA modified IgG solution. Briefly vortex to mix.
3. Incubate the solution for 2 hrs at room temperature.

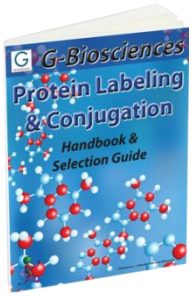
Removal of hydroxylamine using SpinOUT™ GT-600, 5ml columns

1. Centrifuge the SpinOUT™ GT-600 column at 1,000g for 2 minutes to compact the resin.
2. Prepare the Spin-*OUT*™ GT-600 column by removing the top and then bottom caps. Place into an appropriate collection tube.
3. Centrifuge the column at 1,000g for 2 minutes to remove the storage buffer.
4. Place the column in a new collection tube and remove the cap.
5. Add 10 ml of 1 X Optimizer Buffer™ III to the centre of column. .
6. Centrifuge the column at 1,000g for 2 minutes to remove the buffer.
7. Repeat steps 6 and 7 two more times, ensuring the buffer is discarded after each centrifugation.
8. Centrifuge the column at 1,000 g for 2 minutes to remove residual buffer
9. Place the column in a new collection tube and remove the cap.
10. Slowly, apply 1 ml of deacetylation solution treated antibody solution to the center of column.
11. Centrifuge the column at 1,000g for 4 minutes to collect the modified antibody solution free of hydroxylamine.
12. Use the modified antibody for conjugation immediately for conjugation.

RELATED PRODUCTS

Download our Antibody Production and Protein Labeling & Conjugation Handbooks





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