INSTRUCTIONS

Thermo SCIENTIFIC

Hydroxylamine•HCl

26103

0216.2

Number	Description
26103	**Hydroxylamine•HCl**, 25g
	Formula: NH_2OH•HCl
	Molecular Weight: 69.49
	CAS#: 5470-11-1
	Storage: Upon receipt store desiccated at room temperature. Product shipped at ambient temperature.

Introduction

Thermo Scientific Hydroxylamine•HCl is a strong reducing agent that is useful in biochemical crosslinking applications, including the deacetylation of SATA (Product No. 26102) and chemical cleavage of EGS and Sulfo-EGS (Product No. 21565 and 21566, respectively). Hydroxylamine converts carbonyl compounds (aldehydes and ketones) to their oxime derivatives in the presence of a weak base. Therefore, crosslinkers and other compounds that contain a carbonyl within their structure are cleavable with hydroxylamine•HCl.

EGS (Figure 1) and its water-soluble analog, Sulfo-EGS, are homobifunctional, succinimidyl ester, amine-reactive crosslinkers useful for covalent stabilization of polypeptide multimers and protein:protein interactions. Unlike disulfide-containing crosslinkers, EGS and Sulfo-EGS will not cleave by reducing SDS-PAGE conditions but may be cleaved when necessary with hydroxylamine.

SATA (Figure 2) and SATP (Product No. 26100) are modification reagents for addition of sulfhydryl groups to proteins and other molecules containing primary amines. Such sulfhydryl addition is an important step in one popular method for preparing protein conjugates such as antibodies with horseradish peroxidase enzyme. The initial modification results in addition of an acetyl-protected sulfur, enabling storage of the modified protein; to make the sulfur available as a sulhydryl group (-SH) for the final conjugation reaction, hydroxylamine is used to remove the protecting acetyl group.

Hydroxylamine•HCl is more stable to oxidation than the free base form of hydroxylamine; nevertheless, always prepare hydroxylamine solutions immediately before use and store the product desiccated. Hydroxylamine•HCl is soluble in polar solvents such as water, ethanol, methanol, glycerol and propylene glycol.

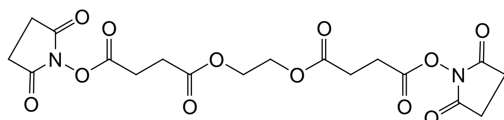

Figure 1. Structure of EGS (Product No. 21565), a crosslinker whose spacer arm may be cleaved with hydroxylamine.

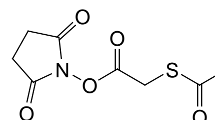

Figure 2. Structure of SATA (Product No. 26102), a modification reagent whose sulfur may be deprotected by deacetylation with hydroxylamine.

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

Procedure for Cleaving EGS Crosslinked Compounds

Note: This procedure is modified from the method used by Abdella, *et al.*[1]

1. Prepare a 2.0M hydroxylamine•HCl solution by adding sufficient hydroxylamine•HCl to a Phosphate Buffer, pH 8.5 and adjusting the pH back to 8.5 with NaOH. Prepare this solution immediately before use.
2. Quickly Warm the hydroxylamine•HCl solution to 37°C and incubate equal volumes of sample and hydroxylamine solution for 3-6 hours with constant mixing.

   Note: Reaction may be performed for 6 hours at room temperature, although cleavage may not be as complete.
3. Immediately dialyze with a Thermo Scientific Slide-A-Lyzer Dialysis Cassette or buffer exchange with a desalting column (see Related Thermo Scientific Products) to remove excess hydroxylamine.
4. Examine products by reducing SDS-PAGE to determine the effectiveness of cleavage. Include appropriate (uncleaved) control lanes.

Deacetylation of a SATA-modified Protein

1. Prepare 0.5M hydroxylamine, 25mM EDTA in phosphate buffered saline (PBS), pH 7.2-8.5.

   Note: To make 50mL of this solution, dissolve 1.74g hydroxylamine•HCl and EDTA (0.475g of tetrasodium salt or 0.365g of disodium salt) in 40mL phosphate buffered saline (PBS), pH 7.2-8.5. Add ultrapure water to a final volume of 50mL and adjust back to the original pH with NaOH. Prepare this solution immediately before use.
2. Combine 1.0mL of SATA-modified protein solution with 100µL of prepared 0.5M hydroxylamine•HCl solution.
3. Mix contents and incubate reaction for 2 hours at room temperature.
4. Use a desalting column to purify the sulfhydryl-modified protein from the hydroxylamine. Desalt into PBS containing 10mM EDTA to minimize disulfide bond formation.
5. 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

21565	**EGS** (ethylene glycol bis[succinimidylsuccinate]), 1g
21566	**Sulfo-EGS** (ethylene glycol bis[sulfosuccinimidylsuccinate]), 50mg
26100	**SATP** (*N*-succinimidyl S-acetylthiopropionate), 50mg
26102	**SATA** (*N*-succinimidyl S-acetylthioacetate), 50mg
22582	**Ellman's Reagent**, 5,5'-Dithio-*bis*-(2-nitrobenzoic acid), 5g
66382	**Slide-A-Lyzer® Dialysis Cassette Kit, 10K MWCO, 3mL**

General References

Abdella, P., *et al.* (1979). A new cleavable reagent for cross-linking and reversible immobilization of proteins. *Biochem Biophys Res Commun* **87(3):**732-42.

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.

Mouton, C.A., *et al.* (1982). A reagent for covalently attaching biotin to proteins via a cleavable connector arm. *Arch Biochem Biophys* **218(1):**101-8.

Product References

Ikeda, T., *et al.* (2003). Multimerization of the receptor activator of nuclear factor-κB ligand (RANKL) isoforms and regulation of osteoclastogenesis. *J. Biol Chem* **278(47):**47217-22.

Tegethoff, S., *et al.* (2003). Tetrameric oligomerization of IκB Kinase γ (IKKγ) is obligatory for IKK complex activity and NF-κB activation. *Mol Cell Biol* **23(6):**2029-41.

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