INSTRUCTIONS

Traut's Reagent

(2-Iminothiolane•HCl)

26101

0414.4

Number	Description
26101	**Traut's Reagent (2-Iminothiolane•HCl)**, 500mg

Molecular Weight: 137.63
CAS#: 4781-83-3
Absorption Maximum (in acetonitrile or 0.1N HCl): 248nm
Extinction Coefficient (in 0.1N HCl): 8840M^{-1} cm^{-1} (+/-5%)

Storage: Upon receipt store product at 4°C with desiccant. Product shipped at ambient temperature.

Introduction

Thermo Scientific Traut's Reagent (2-Iminothiolane or 2-IT) is a cyclic thioimidate compound for thiolation (sulfhydryl addition).[1] Traut's Reagent reacts with primary amines (-NH_2) to introduce sulfhydryl (-SH) groups while maintaining charge properties similar to the original amino group (Figure 1). Once added, sulfhydryl groups may be specifically targeted for reaction in a variety of useful labeling, cross-linking, and immobilization procedures.

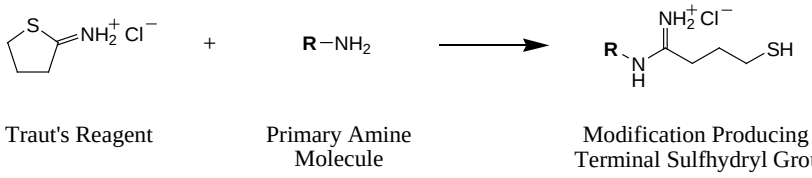

Figure 1. Structure of Traut's Reagent and reaction scheme with molecules containing primary amines.

Important Product Information

A. Reaction Specificity

Traut's Reagent reacts spontaneously and efficiently with primary amines at pH 7-9.[1,2] It also reacts with aliphatic and phenolic hydroxyl groups, especially at high pH; however, the rate of these reactions is 100-fold less than with amino groups[3,4] and will not occur to any appreciable degree when amines are present and reaction times are less than overnight.

B. Reaction Buffers

Various buffers can be used successfully for thiolation with Traut's Reagent. Protein (e.g., antibody) or peptide modifications are most easily performed in phosphate buffered saline (PBS, Product No. 28372) or 0.1M borate buffer adjusted to pH 8, although other buffers devoid of primary amines that maintain solubility of the protein will work equally well. For modification of ribosomal proteins, the buffer most commonly cited in the literature is 50mM triethanolamine•HCl, 1mM $MgCl_2$, 50mM KCl, pH 8.0.[5] Polysaccharide modification (possible only if amines are not also present) is best performed at high pH, such as in 20mM sodium borax buffer, pH 10.[3]

C. Hydrolysis Rate and Reaction Stoichiometry

Traut's Reagent is very stable in acidic or neutral buffers that are devoid of primary amino groups. Even in alkaline conditions, hydrolysis is slow compared to the rate of reaction with primary amines. For example, the half-life of reagent hydrolysis in 50mM triethanolamine buffer at pH 8 is ~1 hour, whereas the half-life of reagent consumption by reaction with primary amines (in the form of 20mM glycine) in the same buffer is ~5 minutes.

Because hydrolysis is slow relative to the amine reaction rate, thiolation with Traut's Reagent does not require as large a molar excess of reagent as other types of modification reagents, such as SATA. In most situations, using a 2-fold molar excess of Traut's Reagent over amines will be sufficient to ensure effective modification. For large proteins that have many

lysine residues (= many amines), adjusting the molar ratio of Traut's Reagent in the reaction allows one to control the level of thiolation. For example, for IgG molecules (150kDa), reaction with a 10-fold molar excess of Traut's Reagent ensures that all antibody molecules will be modified with at least 3-7 sulfhydryl groups. By comparison, nearly all available primary amines (~20 in the typical IgG) could be thiolated using a 50-fold molar reagent excess, but that would be more likely to adversely affect antibody function.

Curiously, Tris and ammonium chloride buffers, both of which contain primary amines, are not particularly reactive with Traut's Reagent at pH 8 (consumption rates of 45 and 35 minutes, respectively). Generally, thiolation of protein primary amines with Traut's Reagent are complete in less than 1 hour, and glycine is a more effective quenching reagent than Tris.

Note: Immediately react sulfhydryl created by Traut's Reagent, because over time the sulfhydryl may recyclize or oxidize to a non-reactive disulfide.

Procedure for Thiolation of Protein with Traut's Reagent

1. Dissolve protein to be thiolated in a non-amine buffer, pH 8.0. **Note:** Include 2-5mM EDTA in the buffer to chelate divalent metals in the solution, which helps to prevent oxidation of sulfhydryls (i.e., formation of disulfide bonds).
2. Depending on protein size and concentration and the level of thiolation desired (see Important Product Information), add a 2- to 20-fold molar excess of Traut's Reagent to the protein in solution. **Note:** Dissolving Traut's Reagent in water or buffer at 2mg/mL results in a 14mM stock solution from which the necessary amount of reagent may be pipetted into the protein solution to initiate the reaction. For example, to modify IgG at a concentration of 10mg/mL using a 10-fold molar excess of Traut's Reagent, add 46µL of the 14mM stock solution to each milliliter of protein solution.
3. Incubate solution for 1 hour at room temperature.
4. Separate thiolated protein from excess Traut's Reagent using a desalting column (e.g., Thermo Scientific Zeba Spin Desalting Column, Product No. 89891) that has been equilibrated with buffer containing 2-5mM EDTA.
5. Sulfhydryl groups may be measured using Ellman's Reagent, Product No. 22582.

Cited References

1. Traut, R.R., *et al*. (1973). Methyl 4-mercaptobutyrimidate as a cleavable cross-linking reagent and its application to the *Escherichia coli* 30S ribosome. *Biochem* **12(17):**3266-73.
2. Jue, R., *et al.* (1978). Addition of sulfhydryl groups to *Escherichia coli* ribosomes by protein modification with 2-iminothiolane (methyl 4-mercaptobutyrimidate). *Biochem* **17(25):**5399-5405.
3. Alagon, A.C. and King, T.P. (1980). Activation of polysaccharides with 2-iminothiolane and its uses. *Biochem* **19:**4341-5.
4. Tarentino, A.L., *et al.* (1993). 2-iminothiolane: a reagent for the introduction of sulfhydryl groups into oligosaccharides derived from asparagine-linked glycans. *Glycobiology* **3:**279-85.
5. Wower, I. and Wower, J. (1981). The use of 2-iminothiolane as an RNA-protein cross-linking agent in *Escherichia coli* ribosomes, and the localisation on 23S RNA of sites cross-linked to proteins L4, L6, L21, L23, L27 and L29. *Nucleic Acids Res* **9(17):**4285-302.

Product References

D'Oro, U., *et al*. (2002). Regulation of constitutive TCR internalization by the ζ-chain. *J Immunol* **169:** 6269-78.

Newton, D.L., *et al.* (2001). Potent and specific antitumor effects of an anti-CD22-targeted cytotoxic ribonuclease: potential for the treatment of non-Hodgkin lymphoma. *Blood* **97:**528-35.

Stanisic, D.I., *et al*. (2003). Analysis of immunological nonresponsiveness to the 19-kilodalton fragment of merozoite surface protein 1 of *Plasmodium yoelii*. *Infec Immunity* **71(10):**5700-13.