FT-UP24221

TCEP

A reducing agent that is odorless and more effective than DTT

Product Description

Catalog number :	UP242214, 1g
Name:	**Tris(2-carboxyethyl)phosphine HCl (TCEP)**
Formula :	CAS : [51805-45-9]
Molecular Weight :	286.65
Storage :	Store at +4°C protected from light and moisture (L) Long term storage recommended at –20°C Harmfull / Irritant; R: 20/22; S: 36

Introduction

Hydrosoluble reagent to protect sulfhydryls or cleave disulfide bridges. Main applications are protein modification for conjugation or labeling, protein activity preservation, and protein reduction before SDS-PAGE electrophoresis.
TCEP replaces advantagely DTT and β-mercaptoethanol, providing **no pungent odor**. It gives a **selective**, complete and **quantitative and quick reduction** (in less than 5 min). An important advantage is also that it is active **at both alkaline and acidic conditions** (unlike DTT), it is **more resistant to air oxidation** and **more hydrophilic**. and it is often **not needed to remove TCEP before modification of protein thiols**. Finally it **does not reduce metals** , that is a great benefit when used in immobilized metal affinity chromatography.

Technical and Scientific Information

Uptima Tris(2-carboxyethyl)phosphine hydrochloride (TCEP), is a high quality reducing reagent used in various biotechnological technics and biology applications:

- reducing agent for proteins before SDS-PAGE analysis
- reducing agent for proteins after chelated-based affinity chromatography
- generating sulfhydryls in proteins from dissulfide bridges before thiol-specific conjugation or labeling
- studies of protein structure and function (Kirley 1989)
- protective reagent for sulfhydryl groups, and acid ascorbic solutions.

- TCEP is **soluble in water up 310mg/ml** (at the opposite to other phosphines), giving a pH of 2.5. It is non-volatile (**odorless**). TCEP is **stable also in basic conditions, at a higher pH** than is DTT and for a longer period of time in buffers without metal chelators such as EGTA. It is also more stable in solutions that contain metal chelators as EGTA (Getz 1999). TCEP is in particular more stable in the presence of Ni^{2+} levels that commonly contaminate proteins eluted from Ni^{2+} affinity columns and that rapidly oxidize DTT (Getz 1999) .
- TCEP is generally **impermeable to cell membrane**s and to the hydrophobic protein core, permitting its use for the selective reduction of disulfides that have aqueous exposure.
- TCEP **resists to air oxidation** (Han 1994, Getz 1999). Oxidation may however be significative under certain conditions, thus it is recommanded to prepare a fresh solution notably if phosphate buffers are used.
- TCEP **efficiently reduces** even the most stable water-soluble alkyl disulfides, over a wide pH range (1.5-9). The strenght of the phosphorus-oxygen bound makes the reaction almost **irreversible**. Kinetics rather than thermodynamics controls the reduction. Unlike DTT, it retains its reducing power **at acid pHs** (pH 5) and **at pH above 7.5**. Complete reduction is usually obtained at room temperature in less

than 5 minutes. The reduction was shown very complete and rapid under several pH, concentration and buffer conditions (Han 1994, Mery 1993), and generally more effective than reduction by DTT (Getz 1999).

- TCEP is **very selective**: it does not react toward other functional groups found in proteins. Hence it allows site specific conjugations.
- TCEP **does not react with buried disulfides**. This is usefull to distinguish thiol-sensitive forms (i.e. Type I aldolase and divalent metal sensitive Type II aldolase (Antil 1997)) and avoid denaturation of proteins while modifying them chemically in surface.
- As it **does not contain sulfhydryl** (at the opposite of DTT). This support the fact that TCEP is less deleterious to maleimide conjugations compared with DTT, which allows only 20-30% of the labeling achieved with TCEP (Getz 1999). This is taken to good account in conjugation process: after reduction step, excess TCEP may not be needed to be removed from protein for subsequent thiol-conjugation step. However, this benefit should be kept with caution, because TCEP has been reported to react with haloacetamides or maleimides under certain conditions (Shafer 2000, Tyagarajan 2003). I.e. TCEP alters in a lower extend (10-20%) iodoacetamides reactions with sufhydryls when at low concentrations, but stronger interference is observed at higher concentrations.

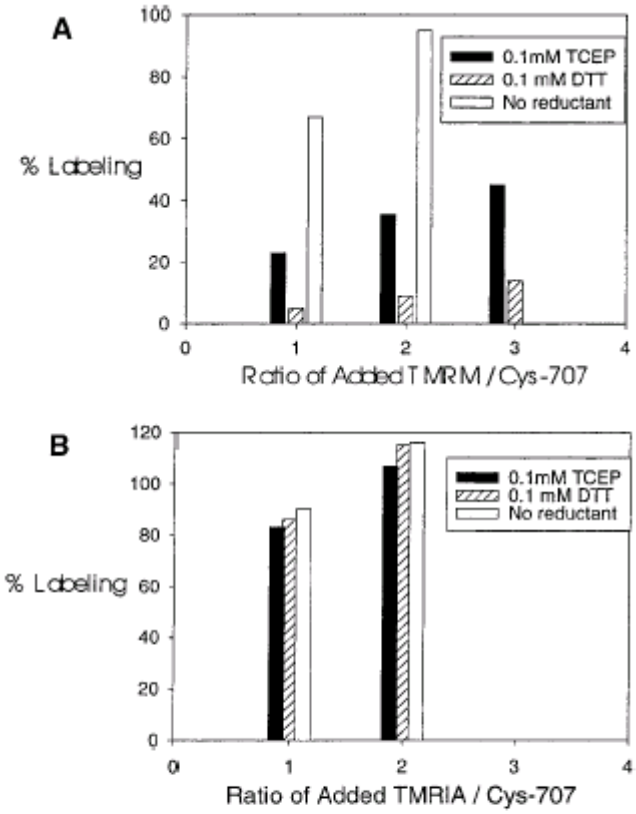

FIG. 3. Cysteine labeling by TMR dye in the presence of 0.1 mM reductant. The percentage modification of the reactive cysteine, Cys-707, of HMM with (A) TMRM or (B) TMRIA is determined at various ratios of added dye/Cys-707. For both dyes, the labeling reaction proceeded with 20 μM Cys-707 for 2 h on ice in the presence of 0.1 mM TCEP (filled bars), 0.1 mM DTT (hatched bars) or no reductant (open bars). Unattached dye was removed by size-exclusion column, and final dye and protein concentrations were determined by absorption as described under Methods. Both DTT and TCEP interfered significantly with maleimide attachment, DTT more so than TCEP (A). At 0.1 mM, neither reductant significantly inhibited iodoacetamide attachment (B).

- TCEP is used at **1-100 molar excess over SS** concentration. Protein reduction can be achieved within 30min with 2-3µM for aquous solutions of 2-10mg protein / ml (Levison 1969).

- Other applications have been reported, besides the widely used application as a reducing agent for proteins and enzymes:

• Studies involving the regulation of gene expression (Chen 1992)
• EPR spectroscopy measurements, where reducants presence is long-standing probelm: TCEP inhibits only half of DTT Nitroxide Spin labels (Getz 1999)
• Protective agent against ionizing radiations in living cell (Shakel 1980, Zhang 1988)
• Enhancement of DNA polymerase activity (Bohn 1974)
• Receptor activity modification by modifying one or more disulfide bonds (Aizenman 1989, Reynolds 1990)
• Inducer of gadd 153 mRNA (Chen 1992)
• Measurement of de/ascorbic acid concentration by substraction method (Lykkesfeldt 2000)
• protein activity preservation, i.e. K_1-ATPase
• protein reduction before SDS-PAGE electrophoresis; However, TCEP has been reported to cause unwanted protein degradation at elevated temperatures used in gel electrophoresis preparations (Getz 1999).

Literature

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Related Products

- Triton X-100, oxidant free, UP521121
- DTT, UP284250
- EDTA, disodium salt, UP036290
- Iodoacetamide, 020486

For any question , please ask Uptima - Interchim, Biology department, Interbiotech
e-mail Uptima@interchim.com; Hotline : +33 (0) 4 70 03 76 06

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