INSTRUCTIONS

TCEP•HCl

20490 20491

0647.5

Number	Description
20490	**TCEP•HCl**, Tris(2-carboxyethyl)phosphine hydrochloride, 1g
20491	**TCEP•HCl**, Tris(2-carboxyethyl)phosphine hydrochloride, 10g
	Molecular Weight: 286.65
	CAS #51805-45-9
	Storage: Upon receipt store at room temperature in sealed container to prevent oxidation. Product shipped at ambient temperature.

Introduction

The Thermo Scientific™ TCEP is a potent, versatile, odorless, thiol-free reducing agent with broad application to protein and other research involving reduction of disulfide bonds (Figure 1). The unique compound is easily soluble and very stable in many aqueous solutions. TCEP reduces disulfide bonds as effectively as dithiothreitol (DTT), but unlike DTT and other thiol-containing reducing agents, TCEP does not have to be removed before certain sulfhydryl-reactive cross-linking reactions.

The ability and virtues of trialkylphosphine compounds to reduce protein disulfide bonds have been known for many years.[1,2] Phosphines are stable in aqueous solution, selectively reduce disulfide bonds, and are essentially nonreactive toward other functional groups commonly found in proteins.[2] However, widespread adoption of trialkylphosphines as reductants for protein research was hindered by their disagreeable odor and poor water solubility. These obstacles were overcome by discovery of tris(2-carboxyethyl)phosphine (TCEP).[3]

TCEP selectively and completely reduces even the most stable water-soluble alkyl disulfides over a wide pH range.[4] Reductions frequently require less than 5 minutes at room temperature. TCEP is non-volatile, odorless, and unlike most other reducing agents, is resistant to air oxidation. Compared to DTT, TCEP is more stable, more effective, and able to reduce disulfide bonds at lower pHs.[5]

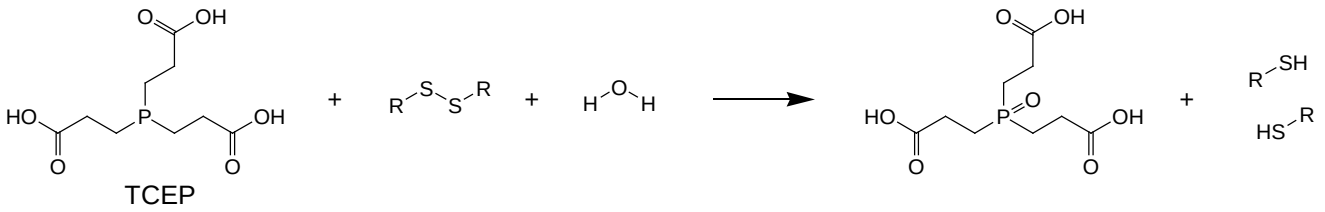

Figure 1. Reduction of organic disulfide bonds with TCEP.

Product Information

Solubility

- The hydrochloride salt (TCEP•HCl, MW 286.65) has a solubility in water of 310g/L (1.08M). Being hydrophilic, TCEP is generally very soluble in aqueous buffers at nearly any pH. Therefore, working concentrations and 10X stock solutions may be readily prepared in most aqueous buffers (see subsequent points about stability). TCEP has only minimal solubility in organic solvents, including methanol and ethanol.

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Stability in Solution

- TCEP is stable in aqueous, acidic, and basic solutions. When TCEP is dissolved directly in water, the resulting pH is approximately 2.5. Studies indicate that no change in concentration of TCEP occurs after 24 hour incubation at room temperature in 100mM HCl, 100mM NaOH, or in any of the following 50mM buffers: Tris•HCl (pH 7.5, 8.5, and 9.5), HEPES (pH 6.8 and 8.2), borate (pH 8.2 and 10.2), and CAPS (pH 9.7 and 11.1).[5] Even after three weeks in these buffers, less than 20% of the TCEP was oxidized.
- TCEP is not particularly stable in phosphate buffers, especially at neutral pH. Experiments indicate that TCEP completely oxidizes within 72 hours in 0.35M phosphate-buffered saline (PBS), pH 7.0. Approximately 50% oxidation occurs in the same amount of time in 0.15M PBS, pH 8.0. Only minimal oxidation occurs in PBS at pH > 10.5 or < 6.0. Therefore, if TCEP is to be used in PBS buffers, prepare the working solution immediately before use.

Effective Reducing pH

- TCEP effectively reduces disulfide bonds over a broad pH range.[5] In one experiment, TCEP completely reduced 2,2´dithiodipyridine (2,2´-DTDP) within 30 seconds at 1.5 < pH < 9.0. Above pH 9.0, only 50% reduction occurred. TCEP is a more effective than DTT at pH < 8.0; TCEP will even reduce oxidized DTT.[5]

Working Concentration

- For most applications, 5-50mM TCEP provides sufficient molar excess to effectively reduce peptide or protein disulfide bonds within a few minutes at room temperature. Han and Han demonstrated complete reduction of 2,2´-DTDP (20µM) by TCEP (30µM) at pH 1.5 to 8.5, within 40 seconds.[5] When molar equivalents of TCEP are used the reaction time is much longer. Mery *et al.* reported that a 1:1 ratio of TCEP to disulfides required nearly one hour to complete the reduction.[6]

Compatible Applications

- Because TCEP does not contain thiols, it does not have to removed from solutions before performing reactions involving maleimide labeling or cross-linking reagents. In most situations, TCEP concentrations < 10-20mM are compatible with maleimide reaction chemistry.
- TCEP may be used as a substitute for DTT or 2-mercaptoethanol (2-ME) in reducing sample loading buffer for SDS-PAGE; use a final concentration of 50mM TCEP (see also Related Thermo Scientific Products). However, because TCEP is charged in solution, it is not compatible for use in isoelectric focusing (IEF), which is the first dimension of 2D electrophoresis.
- One strategy for labeling antibodies involves reducing disulfide bonds in the hinge region of IgG molecules without also reducing those connecting heavy and light chains. This “partial” reduction is often accomplished with 2-mercapto-ethylamine (2-MEA, see Related Thermo Scientific Products). Bläuenstein *et al.*[7] demonstrated that TCEP can be used for this purpose: Add TCEP at a final concentration of 3.8-4.0mM to 10mg/mL IgG in 0.1M phosphate buffer (pH 4.6-7.5); incubate for 20-30 minutes at room temperature, then use a desalting column to remove the TCEP products.

Related Thermo Scientific Products

77720	**Bond-Breaker™ TCEP Solution, Neutral pH**, 5mL
77712	**Immobilized TCEP Disulfide Reducing Gel**, 5mL
20408	**2-Mercaptoethylamine•HCl (2-MEA)**, 6 × 6mg
20290	**Dithiothreitol (DTT),** 5g
20291	**No-Weigh™ Dithiothreitol (DTT)**, 48 tubes × 7.7mg

General References

1. Ruegg, U.T. and Rudinger, J. (1977). Reductive cleavage of cystine disulfides with tributylphosphine. *Methods Enzymol* **47**:111-26.
2. Kirley, T.L. (1989). Reduction and fluorescent labeling of cyst(e)ine-containing proteins for subsequent structural analysis. *Anal Biochem* **180**:231.
3. Burns, J.A., *et al*. (1991). Selective reduction of disulfides by tris-(2-carboxyethyl)-phosphine. *J Org Chem* **56**:2648-50.
4. Levison, M.E., *et al*. (1969). Reduction of biological substances by water-soluble phosphines: Gamma-globulin. *Experentia* **25**:126-7.
5. Han, J.C., *et al*. (1994). A procedure for quantitative determination of tris(2-carboxyethyl)phosphine, an odorless reducing agent more stable and effective than dithiothreitol. *Anal Biochem* **220**:5-10.
6. Mery, J., *et al*. (1993). Disulfide linkage to polyacrylic resin for automated Fmoc peptide synthesis. Immunochemical applications of peptide resins and mercaptoamide peptides. *Int J Peptide Protein Res* **42**:44-52.
7. Blauenstein, P., *et al*. (1995). Experience with the iodine-123 and technetium-99m labeled anti-granulocyte antibody Mab47: a comparison of labeling methods. *Eur J Nuclear Med* **22**:690-8.

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