

Bond-Breaker™ TCEP Solution, Neutral pH

77720

0867.1

Number	Description
77720	Bond-Breaker™ TCEP Solution, Neutral pH , 5 ml, contains a pH neutralized and stabilized aqueous 0.5 M TCEP solution

Storage: Upon receipt store at room temperature. Keep bottle closed when not in use.

Introduction

Bond-Breaker™ TCEP Solution, Neutral pH is a potent, versatile, odorless, thiol-free reducing agent with broad application to protein and other research involving reduction of disulfide bonds (Figure 1). This product is an effective and convenient replacement for β-mercaptoethanol or dithiothreitol in SDS-PAGE sample buffers. The neutral pH formulation avoids exposing proteins to the strong acid associated with TCEP•HCl, which can result in acid hydrolysis and carbohydrate modification, and provides sharp banding patterns.

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 present in proteins.² Trialkylphosphines, however, were hindered by their instability in water and their disagreeable odor. These obstacles were overcome by discovery of tris(2-carboxyethyl)phosphine (TCEP).³⁻²⁵

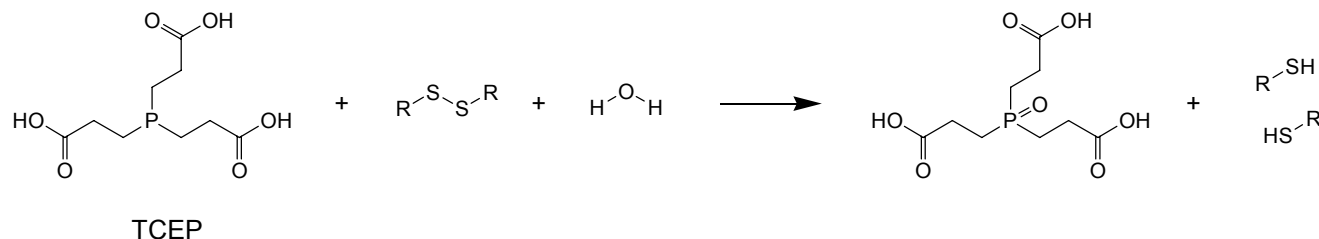


Figure 1. Reduction of organic disulfide bonds using TCEP.

Procedure for Polyacrylamide Gel Electrophoresis

1. Add Bond-Breaker™ TCEP Solution, Neutral pH to a final concentration of 50 mM (1 to 10 dilution) in 2X SDS sample buffer for Tris-glycine gels (25 mM Tris, 20% glycerol, 4% SDS, 0.005% bromophenyl blue, pH 6.8).
2. Mix equal volumes of sample and 2X concentrate reducing sample buffer in a microcentrifuge tube and heat to 95°C in a boiling water bath for 5 minutes.
3. Allow samples to cool and then load reduced sample onto a gel. To remove any insoluble protein aggregates, briefly centrifuge the sample after heating and before loading.

Related Pierce Products

77712	Immobilized TCEP Disulfide Reducing Gel , 5 ml
24615	Imperial™ Protein Stain , 1 L, coomassie R-250 stain
24590	GelCode™ Blue Stain Reagent , 500 ml, coomassie G-250 stain
24612	SilverSNAP® Stain Kit II

Warranty: Pierce Biotechnology products are warranted to meet stated product specifications and to conform to label descriptions when stored and used properly. Unless otherwise stated, this warranty is limited to one year from date of sale when used according to product instructions. Pierce Biotechnology's sole liability for the product is limited to replacement of the product or refund of the purchase price. Unless otherwise expressly authorized in writing by Pierce Biotechnology, Pierce products are supplied for Research Use Only and are intended to be used by a technically qualified individual. Pierce Biotechnology's quality system is certified to ISO 9001. Pierce Biotechnology products are not produced in accordance with FDA's current Good Manufacturing Practices. Pierce Biotechnology strives for 100% customer satisfaction. If you are not satisfied with the performance of a Pierce Biotechnology product, please contact Pierce Biotechnology or your local distributor.

24600	SilverSNAP[®] Stain for Mass Spectrometry
24597	Color Silver Stain Kit
24582	E-Zinc[®] Reversible Stain Kit
24614	Silver Stain Rescue Reagent
25200-25244	Precise[™] Protein Gels (see catalog or web site for a complete listing)
26681	BlueRanger[®] Prestained Protein Molecular Weight Marker Mix , 48 single-dose tubes
26691	TriChromRanger[®] Prestained Protein Molecular Weight Marker Mix , 48 single-dose tubes
20408	2-Mercaptoethylamine•HCl (2-MEA) , 6 x 6 mg
20290	DTT, Cleland's Reagent (dithiothreitol) , 5 g
20291	No-Weigh[™] Dithiothreitol (DTT) , 48 tubes × 7.7mg

References

- Ruegg, U.T and Rudinger, J. (1977). Reductive cleavage of cystine disulfides with tributylphosphine. *Methods Enzymol.* **47**:111-26.
- Kirley, T.L. (1989). Reduction and fluorescent labeling of cyst(e)ine-containing proteins for subsequent structural analysis. *Anal. Biochem.* **180**:231.
- Burns, J.A., *et al.* (1991). Selective reduction of disulfides by tris-(2-carboxyethyl)-phosphine. *J. Org. Chem.* **56**:2648-50.
- Han, J., *et al.* (1999). Tris[2-carboxyethyl]phosphine – A reducing agent with versatile applications including cleavage of disulfide bonds and quantitation of numerous oxidants. *Previews* **2(4)**:16-21.
- Han, J., *et al.* (1993). Modification of catalytic properties of chicken liver fructose 1,6-bisphosphatase by allicin. *Biochem. Mol. Biol. Int.* **31**:1007-1015.
- Han, J.C. and Han, G.Y. (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.
- Mery, J., *et al.* (1993). Disulfide linkage to polyacrylic resin for automated Fmoc peptide synthesis, immunochemical applications of peptide resin and mercaptoamide peptide. *Int. J. Pept. Protein Res.* **42**:44-52.
- Gray, W.R. (1993). Disulfide structures of highly bridged peptides: a new strategy for analysis. *Protein Sci.* **2**:1732-48.
- Fisher, W.H., *et al.* (1993). *In situ* reduction suitable for matrix-assisted laser desorption/ionization and liquid secondary ionization using tris(2-carboxyethyl)phosphine. *Rapid Commun. Mass Spectrom.* **7**:225-8.
- Gozlan, H., *et al.* (1994). Anoxic LTP is mediated by the redox modulatory site of the NMDA receptor. *J. Neurophys.* **72**:3017-22.
- Gozlan, H., *et al.* (1995). In CA1 hippocampal neurons, the redox state of NMDA receptors determine LTP expressed by NMDA but not by AMPA receptors. *J. Neurophys.* **73**:2612-17.
- Bieri, S., *et al.* (1995). Disulfide bridges of a cysteine-rich repeat of the LDL receptor ligand-binding domain. *Biochemistry* **34**:13059-65.
- Tam, J.P., *et al.* (1995). Peptide synthesis using unprotected peptides through orthogonal coupling methods. *Proc. Natl. Acad. Sci. USA* **92**:12485-9.
- Blauenstein, P., *et al.* (1995). Experience with the iodine-123 and technetium-99m labelled anti-granulocyte antibody MAb47: a comparison of labelling methods. *Eur. J. Nucl. Med.* **22**:690-8.
- Gorman, J.J., *et al.* (1996). Use of 2,6-dihydroxyacetophenone for analysis of fragile peptides, disulphide bonding and small proteins by matrix-assisted laser desorption/ionization. *Rapid Commun. Mass Spectrom.* **10**:529-36.
- Hirsch, J.C., *et al.* (1996). Enhanced NMDAR-dependent epileptiform activity is controlled by oxidizing agents in a chronic model of temporal lobe epilepsy. *J. Neurosci.* **76**:4185-9.
- Quesada, O., *et al.* (1996). Redox sites of NMDA receptors can modulate epileptiform activity in hippocampal slices from kainic acid-treated rats. *Neurosci. Lett.* **212**:171-4.
- Kirsch, T., *et al.* (1996). Cloning, high-yield expression in *Escherichia coli*, and purification of biologically active HIV-1 Tat protein. *Protein Express. Purif.* **8**:75-84.
- Haniu, M., *et al.* (1996). Glial cell line-derived neurotrophic factor: selective reduction of the intermolecular disulfide linkage and characterization of its disulfide structure. *Biochemistry* **35**:16799-05.
- White, C.E., *et al.* (1996). The fifth epidermal growth factor-like domain of thrombomodulin does not have an epidermal growth factor-like disulfide bonding pattern. *Proc. Natl. Acad. Sci.* **93**:10177-82.
- Xiao, Y., *et al.* (1997). Involvement of disulfide bonds in the renal sodium/phosphate co-transporter NaPi-2. *Biochem. J.* **323**:401-8.
- Wu, J. and Watson, J.T. (1997). A novel methodology for assignment of disulfide bond pairings in proteins. *Protein Sci.* **6**:391-8.
- Bernard, C.L., *et al.* (1997). Redox modulation of synaptic responses and plasticity in rat CA1 hippocampal neurons. *Exp. Brain Res.* **113**:343-52.
- Riddles, P.W., *et al.* (1979). Ellman's reagent: 5,5'-dithiobis (2-nitrobenzoic acid) - A reexamination. *Anal. Biochem.* **94**:75-81.
- Cavallito, C.G., *et al.* (1944). Allicin, the antibacterial principle of *Allium salivum*. II. Determination of the chemical structure. *J. Am. Chem. Soc.* **66**:1952-4.

Current versions of product instructions are available at www.piercenet.com. For a faxed copy, call 800-874-3723 or contact your local distributor.

©Pierce Biotechnology, Inc., 7/2005. Printed in the USA.