INSTRUCTIONS

# Bond-Breaker™ TCEP Solution, Neutral pH

Pub. No. MAN0011394
Rev. B.0
Pub Part No. 2160867.3

77720

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

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

## Introduction

Thermo Scientific 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]

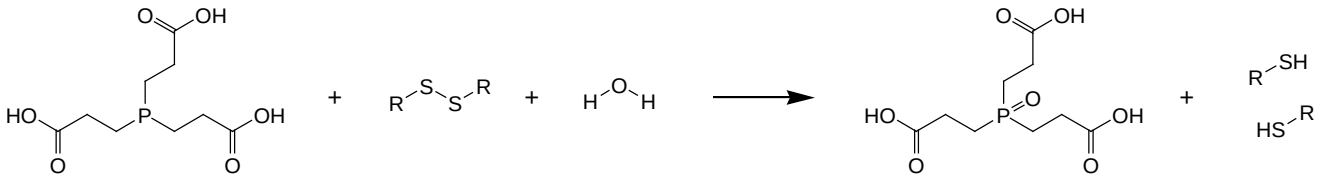


**Figure 1.** Reduction of organic disulfide bonds using TCEP.

## Procedure for Polyacrylamide Gel Electrophoresis

1. Add Bond-Breaker TCEP Solution, Neutral pH to a final concentration of 50mM (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.

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

## Related Products

| | |
|---|---|
| **77712** | **Immobilized TCEP Disulfide Reducing Gel**, 5mL |
| **24615** | **Imperial™ Protein Stain**, 1L, coomassie R-250 stain |
| **LC6060** | **SimplyBlue™ SafeStain** |
| **24612** | **Pierce™ Silver Stain Kit** |
| **24600** | **Pierce Silver Stain Kit for Mass Spectrometry** |
| **24582** | **Pierce Zinc Reversible Stain Kit** |
| **24614** | **Pierce Silver Stain Rescue Reagent** |
| **XP04200BOX** | **Novex™ Tris-Glycine protein gels** (see thermofisher.com/proteingels for a complete listing) |
| **LC5615** | **iBright™ Prestained Protein Ladder** |
| **26619** | **PageRuler™ Plus Prestained Protein Ladder** |
| **20408** | **2-Mercaptoethylamine•HCl (2-MEA)**, 6 × 6mg |
| **20291** | **Dithiothreitol (DTT)**, **No-Weigh™ format,** 48 tubes × 7.7mg |

### Cited 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. 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.
5. Han, J., *et al*. (1993). Modification of catalytic properties of chicken liver fructose 1,6-bisphosphatase by allicin. *Biochem Mol Biol Int* **31:**1007-15.
6. 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.
7. 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.
8. Gray, W.R. (1993). Disulfide structures of highly bridged peptides: a new strategy for analysis. *Protein Sci* **2:**1732-48.
9. 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.
10. Gozlan, H., *et al*. (1994). Anoxic LTP is mediated by the redox modulatory site of the NMDA receptor. *J Neorophys* **72:**3017-22.
11. 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.
12. Bieri, S., *et al*. (1995). Disulfide bridges of a cysteine-rich repeat of the LDL receptor ligand-binding domain. *Biochemistry* **34:**13059-65.
13. Tam, J.P., *et al*. (1995). Peptide synthesis using unprotected peptides through orthogonal coupling methods. *Proc Natl Acad Sci USA* **92:**12485-9.
14. 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.
15. 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.
16. 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.
17. 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.
18. 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.
19. 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.
20. 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.
21. Xiao, Y., *et al*. (1997). Involvement of disulfide bonds in the renal sodium/phosphate co-transporter NaPi-2. *Biochem J* **323:**401-8.
22. Wu, J. and Watson, J.T. (1997). A novel methodology for assignment of disulfide bond pairings in proteins. *Protein Sci* **6:**391-8.
23. Bernard, C.L., *et al*. (1997). Redox modulation of synaptic responses and plasticity in rat CA1 hippocampal neurons. *Exp Brain Res* **113:**343-52.
24. Riddles, P.W., *et al*. (1979). Ellman's reagent: 5,5´-dithiobis (2-nitroben-zoic acid) - A reexamination. *Anal Biochem* **94:**75-81.
25. 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.

For research use only. Not for use in diagnostic procedures.

This product (“Product”) is warranted to operate or perform substantially in conformance with published Product specifications in effect at the time of sale, as set forth in the Product documentation, specifications and/or accompanying package inserts (“Documentation”) and to be free from defects in material and workmanship. Unless otherwise expressly authorized in writing, Products are supplied for research use only. No claim of suitability for use in applications regulated by FDA is made. The warranty provided herein is valid only when used by properly trained individuals. Unless otherwise stated in the Documentation, this warranty is limited to one year from date of shipment when the Product is subjected to normal, proper and intended usage. This warranty does not extend to anyone other than the original purchaser of the Product (“Buyer”).

**No other warranties, express or implied, are granted, including without limitation, implied warranties of merchantability, fitness for any particular purpose, or non infringement. Buyer’s exclusive remedy for non-conforming Products during the warranty period is limited to replacement of or refund for the non-conforming Product(s).**

There is no obligation to replace Products as the result of (i) accident, disaster or event of force majeure, (ii) misuse, fault or negligence of or by Buyer, (iii) use of the Products in a manner for which they were not designed, or (iv) improper storage and handling of the Products.

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



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