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A Geno Technology, Inc. (USA) brand name

TCEP Reducing Resin

Reduction of Peptides & Protein

(Cat. # 786-822)



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INTRODUCTION

G-Biosciences TCEP Reducing Resin consists of Tris [2-carboxyethyl] phosphine hydrochloride covalently coupled to 4% crosslinked beaded agarose. The resin allows for the efficient reduction of peptide and protein disulfide bonds.

The advantage of immobilizing the TCEP to resin is that the reducing agent can rapidly be removed from the reaction and limit downstream interference.

TCEP is a water-soluble, odorless, non-toxic and stable protein reductant. This potent reducing agent is a popular alternative to β -mercaptoethanol and DTT (dithiothreitol) use for most applications. The reduction potency of TCEP is twice as high as that of DTT, and TCEP is effective in reducing proteins over a wider range of pH conditions, including lower acidic pH ranges (pH 2-11), compared to other reductants.

TCEP reduces stable disulfide bonds in less than 5 minutes at room temperature and is compatible with the protein alkylation reactions.

ITEM(S) SUPPLIED (Cat. # 786-822)

Description	Size
TCEP Reducing Resin	1ml resin

STORAGE CONDITIONS

Shipped at ambient temperature. Upon receipt, store at 4°C, do not freeze.

IMPORTANT INFORMATION

- TCEP Reducing Resin is compatible in a wide range of buffers and works effectively at pH 4-9 and between a temperature range of 0-95°C.
- To increase accessibility of internal disulfide bonds, a denaturant, such as Guanidine.HCl can be added to the reaction.
- Below is a guide to incubation times, however these are ultimately dependent on the protein type, concentrations and conditions used, so optimization may be required.

NOTE: Optimization of reaction times can be monitored by assaying various time points with Ellman's Reagent (Cat. # BC87)

Protein Concentration (mg/ml)	Incubation Time (min)
<0.1	15
0.1-0.5	30
0.5-0.9	45
>1	60

- Metal ions will interfere with reduction. Avoid the use of metal spatulas and other metal items.
- The use of 5-20mM EDTA in the reaction buffer offers 2 advantages:
 - Prevent oxidation of generated sulfhydryls.
 - Chelate interfering divalent metal ions, including Cu^{2+} , Zn^{2+} and Mg^{2+}

PROTOCOL 1: SPIN FORMAT REDUCTION (50-750 μl Samples)

1. Add 1-2 volumes of TCEP Reducing Resin to a spin column, where 1 volume is equal to 1 volume of sample. For example, if reducing 30 μl sample then add 30-60 μl TCEP Reducing Resin.

NOTE: For larger protein samples (300-750 μl), perform in batch method (see below) and transfer to spin column after incubation step (Step 5).

2. Place spin column in a collection tube and centrifuge at 1,000g for 0.5-1 minute and remove and discard the supernatant,
3. Wash the resin with 1-2 volumes of sample buffer.
4. Add the protein/peptide solution to the column, seal the column and vortex to mix.
5. Incubate for the desired time (see table) and temperature with gentle rocking or shaking.
6. Centrifuge at 1,000g for 0.5-1 minute and recover the supernatant, the reduced protein/peptide.
7. The level of reduction can be determined with Ellman's Reagent or analysis by non-reducing SDS PAGE.

PROTOCOL 2: GRAVITY FLOW FORMAT REDUCTION (>250 μl Samples)

1. Pour 1-2 volumes of TCEP Reducing Resin into a suitable gravity flow column that is sealed at the bottom. 1 volume is equal to 1 volume of sample. For example, if reducing 30 μl sample then add 30-60 μl TCEP Reducing Resin.

NOTE: Avoid the introduction of air bubbles as these will decrease the flow rate and reduce capacity.

2. Wash the resin with 1-2 volumes of sample buffer or ultrapure water.
3. Add the protein/peptide solution to the column, seal the bottom of the column once the entire sample has entered the resin bed.
4. Incubate for the desired time (see table) and temperature with gentle rocking or shaking.
5. Recover the sample from the column using the incubation buffer. Collect fractions using an appropriate fraction size. Determine the fractions containing protein or peptide with UV absorbance of a protein assay.
6. The level of reduction can be determined with Ellman's Reagent or analysis by non-reducing SDS PAGE.

PROTOCOL 3: BATCH FORMAT REDUCTION (20-750µl Samples)

1. Add 1-2 volumes of TCEP Reducing Resin to a microcentrifuge tube, where 1 volume is equal to 1 volume of sample. For example, if reducing 30µl sample then add 30-60µl TCEP Reducing Resin.
2. Centrifuge at 1,000g for 1 minute and remove and discard the supernatant,
3. Wash the resin with 1-2 volumes of sample buffer.
4. Add the protein/peptide solution to the washed resin and vortex to mix.
5. Incubate for the desired time (see table) and temperature with gentle rocking or shaking.
6. Centrifuge at 1,000g for 1 minute and recover the supernatant, the reduced protein/peptide.
NOTE: *Some sample loss will occur, but if necessary it can be reversed by washing resin with buffer. The resulting sample will be diluted.*
7. The level of reduction can be determined with Ellman's Reagent or analysis by non-reducing SDS PAGE.

APPENDIX 1: ASSAY REDUCING ACTIVITY OF TCEP REDUCING RESIN

Additional Materials Required

- Ellman's reagent (Cat. # BC87)
- TCEP (Cat. # 786-030)

Protocol

1. Prepare a 10mM Ellman's reagent solution by adding 40mg to 10ml 100mM Tris buffer at pH7.5.
NOTE: *The Ellman's reagent may take an hour to dissolve at room temperature.*
2. Prepare free TCEP Standards
 - a. 20mM TCEP: Dissolve 57.5mg in 10ml deionized water
 - b. 4mM TCEP: Add 2ml 20mM TCEP to 8ml deionized water
 - c. 2mM TCEP: Add 5ml 4mM TCEP to 4ml deionized water
 - d. 1mM TCEP: Add 5ml 2mM TCEP to 4ml deionized water
 - e. 0.5mM TCEP: Add 5ml 1mM TCEP to 4ml deionized water
3. Combine 900µl Ellman's reagent with 10µl each standard or 10µl TCEP Reducing Resin and measure the absorbance at 412nm.
NOTE: *Wait 1-2 minutes before reading the TCEP Reducing Resin to settle to the bottom of the cuvette.*
4. Prepare a standard curve and determine the activity of the TCEP Reducing Resin from the curve.
NOTE: *As the 50% slurry is used, the actual activity is approximately the estimated value determined above.*

APPENDIX 2: CALCULATE LEVEL OF REDUCTION WITH ELLMAN'S REAGENT

1. Make 10mM DTNB stock solution by dissolving 40mg DTNB in 10ml DMSO. The stock solution can be stored at 4°C for 3 months. Dilute the stock solution 100 fold with 0.1M Tris-HCl pH 7.5 to make 0.1mM DTNB working solution.
2. Aliquot 950µl of 0.1mM DTNB work solution to each 1.5ml centrifuge tube. Add 50µl test sample and mix by brief vortexing. Set a blank by adding 50µl of 0.1M Tris-HCl pH 7.5 to 950µl of 0.1mM DTNB work solution.

NOTE: The test sample may need to be diluted before applied to the assay and the dilution factor should be recorded. The 50µl test sample applied to the assay reaction should have a sulfhydryl concentration less than 0.5mM. Concentrations exceeding 0.5mM free sulfhydryl will result in high absorbance values and less accurate estimation of the concentration based on the extinction coefficient.

3. Incubate 2 minutes at room temperature.
4. Measure the absorbance of the test sample with a spectrophotometer against blank at 412nm.

Calculation

Calculate the concentration of free sulfhydryls in the sample from the molar extinction coefficient of NTB ($14.15 \text{ mM}^{-1} \text{ cm}^{-1}$) as follow:

$$\text{mM free sulfhydryls} = \text{Absorbance} / (\text{path length} \times 14.15) \times 20 \times \text{dilution factor}$$

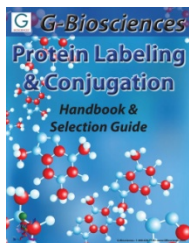
NOTE: Path length is the cuvette path length in centimeters (cm)
20 is the dilution factor of 50µl sample to 1ml assay volume

TROUBLESHOOTING

Issue	Cause	Solution
Poor or incomplete reduction of sample	Insufficient TCEP Reducing Resin Used	Use recommend amount, increase to 2 volumes
	Incubation time too short	For proteins, review and optimize incubation times. For peptides, add a short incubation step.
	Steric hindrance blocking access to disulfide bonds	Use the denaturant guanidine.HCl to open up protein.
	Incubation too long	Do not exceed 2 hours
Reducing activity of resin is low	Product passed its shelf life	Purchase new product
	Product stored in different buffer than originally supplied in	
	Product exposed to metal ions	
Loss of protein/peptide	Batch method was used	Use spin column to increase recovery
	Wrong fractions collected in gravity flow method	Monitor fractions for protein
	Protein bound non-specifically to resin	Modify the buffer and use higher salt concentration, different pH or supplement in DMSO (10-30%)

RELATED PRODUCTS

Download our Protein Labeling & Conjugation Handbook.



<http://info.gbiosciences.com/complete-protein-labeling-conjugation-handbook>

For other related products, visit our website at www.GBiosciences.com or contact us.

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