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A Geno Technology, Inc. (USA) brand name

FOCUS™ Protein Reductant

INTRODUCTION

FOCUS™ Protein Reductant is an odorless, non-toxic, and stabilized solution of TCEP [Tris(2-carboxyethyl) phosphine] for protein reduction, supplied with reductant buffer. As compared to DTT and β-mercaptoethanol, the TCEP is more stable, more effective and able to work over a wide range of pH, including lower acidic pH. It reduces completely even the most stable disulfide bonds in less than 5 minutes at room temperature. The use of the TCEP is compatible with the alkylation reaction of the SH-groups for 2D analysis. Unlike DTT and other commonly used reductants, the TCEP does not compete with the alkylation reagent iodoacetamide.

The kit is supplied with a proprietary Reductant Buffer necessary for an efficient reduction of disulfide bonds while minimizing re-oxidation of the competing thiol pairs in protein samples. The reagents provided with the kit are sufficient for 100 preps, 1-2ml each.

ITEM(S) SUPPLIED	Cat# 786-230
FOCUS™-Protein Reductant	2 x 1.0ml
Reductant Buffer	1.5ml

STORAGE CONDITION

The kit is shipped at ambient temperature. Upon arrival, store the kit components at 4°C. The kit components are good for one year, when stored and used as recommended.

PROTOCOL

Protein reduction and alkylation may be performed in the same reaction tube, or IPG-Strips in two separate steps. We recommend reduction prior to alkylation as reducing agents added after iodoacetamide treatment will react with excess iodoacetamide.

NOTE: *If a precipitate or crystal formation is seen in the Reductant Buffer, warm to room temperature and vortex to dissolve.*

1. ***Protein Reduction***: Add 2.5µl Reductant Buffer for every 500µl 0.2-1mg/ml protein solution and vortex for 10 seconds.

2. Add 10µl FOCUS™ Protein Reductant for every 500µl 0.2-1mg/ml protein solution. Incubate at 55°C for 1 hour.

3. At the end of incubation, the protein solution is ready for next use or for alkylation of the thiols.

PROTOCOL FOR ALKYLATION

1. Iodoacetamide is unstable and light-sensitive. To preserve activity of iodoacetamide, prepare the iodoacetamide solutions immediately before use and perform the alkylation step in the dark.
2. Perform alkylation with limiting quantities of iodoacetamide at a slightly alkaline pH (pH8-9) to ensure alkylation is exclusive to cysteine residues. Excess or non-buffered iodoacetamide may result in alkylation of lysines, N-termini, methionines, histidines, aspartates and glutamates. The supplied alkylation buffer should be added to the solutions to be alkylated to ensure exclusive cysteine residue alkylation.
4. Immediately prior to use, weigh 50mg iodoacetamide in to a microcentrifuge tube. Add 0.4ml deionized water and vortex to dissolve to generate a 0.4M solution. Protect the solution from light.
5. Add 25µl 0.4M iodoacetamide for every 500µl 0.2-1mg/ml protein solution. Incubate at room temperature for 30-60 minutes, protected from light. Discard any unused iodoacetamide solution.

6. The sample is now ready for proteolytic digestion, 2D gel analysis or other downstream application

RELATED PRODUCTS

1. ***PAGEPerfect™ (Cat. #786-123)*: *A kit for preparing samples for PAGE electrophoresis.***
2. ***Perfect-FOCUS™ (Cat # 786-124)*: *A kit for preparing samples for 2D gels.***
3. ***FASTsilver™ (Cat # 786-30)*: *For staining proteins and Nucleic acids in acrylamide gels.***
4. ***FOCUS™ Fast Silver (Cat # 786-240)*: *Sufficient for 25 gels.***
5. ***NI Protein Assay Kit (Cat. #786-005)*: *A protein assay that is free from interference of common laboratory agents including reducing agents, detergents, dyes, EDTA etc.***
6. ***FOCUS™ Protease Arrest (Cat # 786-108F)*: *A protease cocktail specifically developed for sample preparation for 2D-studies and provides 95-98% inhibition of protease activity.***

NOTE*: *For other related products, visit our web site at www.GBiosciences.com or contact us.

Last saved: 8/25/11 IA