



FOCUS™ Protein Reduction-Alkylation

INTRODUCTION

FOCUS™ Protein Reduction-Alkylation kit offers a simple two-step method for reduction and alkylation of protein samples for 2D gel analysis. The disulfide bonds are reduced with a highly reactive and stable TCEP [Tri- (2-carboxyethyl)phosphine] followed by blocking of the thiols by alkylation. The kit is supplied with proprietary buffers and reagents necessary for an efficient reduction and alkylation of the disulfide bridges in protein samples for 2D gel analysis while minimizing re-oxidation of the thiols. The reagents provided with the kit are sufficient for 100 preps, 1-2ml each.

Suitable for: iso-electric focusing buffers, equilibration of IPG-Strips following iso-electric focusing for second dimension SDS-PAGE step, or any application where reduction of disulfide and alkylation of thiols are needed.

ITEM INCLUDED Cat # 786-231

FOCUS™ -Protein Reductant	2.0ml
Reductant Buffer	1.5ml
Iodoacetamide (Cat# 786-228)	5.0 g
Alkylation Buffer	1.5ml

STORAGE CONDITION

Shipped at ambient temperature. Upon arrival, store the kit components in - 20°C. When stored and used properly, the kit components are good for one year.

PROTOCOL

Protein reduction and alkylation may be performed in the same reaction tube, or IPG-Strips in two separate steps - reduction followed by alkylation of the thiols.

Reduction of Disulfide bonds

***Note:** If you notice any precipitate or crystal formation in the Reductant Buffer after it is thawed, warm it to room temperature and vortex to dissolve.*

1. Add Reductant Buffer (5µl/ml) into the reaction/protein solution. Vortex or agitate the reaction.
2. Add FOCUS™ -Protein Reductant (10µl/ml) in the reaction. Vortex or agitate the reaction and incubate for 15 minutes at room temperature.
3. At the end of incubation, the reaction/protein solution is ready for next use or for alkylation of the thiols.

Alkylation of thiols

Following reduction of the disulfide bonds, the protein is ready for alkylation of the thiols produced by the reduction step.

***NOTE-** For efficient alkylation, the reaction should be performed at ~ pH 8-9. If necessary, add an aliquot of a concentrate solution of an alkaline buffer in the reaction to adjust pH ~ 8-9.*

Prepare Fresh Iodoacetamide Alkylation reagent (0.5M) - Weigh 50mg Iodoacetamide in a microfuge tube. Add 0.5 ml pure water. Vortex to dissolve the Iodoacetamide.

***Note:** If you notice any precipitate or crystal formation in the Alkylation Buffer after it is thawed, warm it to room temperature and vortex to dissolve.*

1. Add Alkylation Buffer (5µl/ml) into the reaction/protein solution. Vortex or agitate the reaction.



2. Add freshly prepared Iodoacetamide Alkylation reagent (30µl/ml). Vortex or agitate the reaction. Discard any unused Iodoacetamide Alkylation reagent.
3. Incubate the reaction for 15 minutes at room temperature. At the end of incubation, the reaction/protein solution is ready for next use.

Note: *Depending on the nature of the protein, some proteins' solubility may be reduced after alkylation.*

RELATED PRODUCTS

1. **PAGE Perfect (Cat. #786-123)**: A kit for preparing sample for PAGE electrophoresis.
2. **Perfect-FOCUS (Cat # 786-124)**: A kit for preparing sample for 2D gels.
3. **FAST-Silver (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. **RAPID-Stain (Cat # 786-31)**: For staining protein in gels. RAPID-Stain only stains proteins, leaving clear background with high band visibility. Generally does not require de-staining.
7. **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.
8. **Protease Arrest (Cat # 786-108)**: A cocktail of protease inhibitors for use during protein extraction and purification. Protease Arrest inhibits a broad spectrum of serine, cysteine and metalloproteases.

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

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