



FOCUS™ -Protein Alkylation

INTRODUCTION

FOCUS™ -Protein Alkylation Kit contains ultra-pure proteomic grade Iodoacetamide reagent for protein alkylation. The kit is supplied with a proprietary buffer necessary for an efficient alkylation of the thiols 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.

ITEMS SUPPLIED Cat # 786-232

Iodoacetamide	5gm
Alkylation Buffer	1.5ml

STORAGE CONDITION

Shipped at ambient temperature. Upon arrival, store at - 20°C. When stored and used properly, the kit components are good for use for 1 year.

PROTOCOL

Alkylation of Thiols

Following reduction of 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 microcentrifuge tube. Add 0.5 ml pure water. Vortex to dissolve 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 for 10 seconds.
2. Add freshly prepared Iodoacetamide Alkylation reagent (30µl/ml) into the reaction. 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 samples for PAGE electrophoresis.
2. **Perfect-FOCUS (Cat # 786-124):** A kit for preparing samples 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.
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..

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

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