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A Geno Technology, Inc. (USA) brand name

FOCUS™ Protein Alkylation

(Cat. # 786-232)



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INTRODUCTION

FOCUS™ Protein Alkylation kit contains ultrapure proteomic grade iodoacetamide reagent for protein alkylation. Iodoacetamide is a commonly used alkylation agent for blocking thiols of proteins. Alkylation by iodoacetamide of free cysteines, following their reduction, results in the covalent coupling of a carbamidomethyl group (57.07Da) and prevents formation of disulfide bonds/ bridges. We recommend reduction prior to alkylation as reducing agents added after iodoacetamide treatment will react with excess iodoacetamide. The kit is supplied with a proprietary buffer necessary for an efficient alkylation of the thiols. The reagents provided with the kit are sufficient for 100 preps, 1-2ml each.

ITEM(S) SUPPLIED (Cat. # 786-232)

Description	Size
Iodoacetamide	5gm
Alkylation Buffer	1.5ml

STORAGE CONDITION

The kit is shipped at ambient temperature, upon arrival, store at 4°C. When stored and used properly, the kit components are good for use for one year.

IMPORTANT INFORMATION

- 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.
- 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.

PROPERTIES

Chemical name: 2-Iodoacetamide

Formula: ICH₂CONH₂

Molecular Weight: 184.96

CAS No: 144-48-9

PROTOCOL

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

1. Add 0.5µl Alkylation Buffer for every 100µl 0.2-1mg/ml protein solution and vortex for 10 seconds.

Protein Reduction

1. Add 5µl 200mM TCEP•HCl (Tris (2-carboxyethyl) phosphine hydrochloride) for every 100µl 0.2-1mg/ml protein solution. Incubate at 55°C for 1 hour.

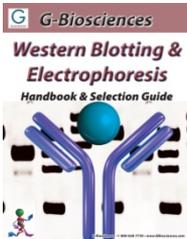
NOTE: Available from G-Biosciences; Cat. # 786-030 TCEP•HCl

Protein Alkylation

1. 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.
2. Add 5µl 0.4M Iodoacetamide for every 100µl 0.2-1mg/ml protein solution. Incubate at room temperature for 30-60 minutes, protected from light. Discard any unused iodoacetamide solution.
3. The sample is now ready for proteolytic digestion, 2D gel analysis or other downstream application

RELATED PRODUCTS

Download our Western Blotting and Electrophoresis Handbook.



<http://info.gbiosciences.com/complete-western-blot-handbook--selection-guide>

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

Last saved: 8/29/2013 13 SM



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