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A Geno Technology, Inc. (USA) brand name

FOCUS™ Protein Reduction-Alkylation

(Cat. # 786-231)

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 [Tris(2-carboxyethyl) phosphine] followed by blocking of the thiols by iodoacetamide 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. 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 reoxidation of the thiols. The reagents provided with the kit are sufficient for 100 preps, 1-2ml each.

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

ITEM(S) SUPPLIED (Cat. # 786-231)

Description	Size
FOCUS™ Protein Reductant	2 x 1.0ml
Reductant Buffer	1.5ml
Iodoacetamide	5g
Alkylation Buffer	1.5ml

STORAGE CONDITION

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

IMPORTANT INFORMATION

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.

Chemical name:	2-Iodoacetamide	[Tris(2-carboxyethyl) phosphine] (TCEP•HCl)
Formula:	ICH_2CONH_2	$C_9H_{15}O_6P$ HCl
Molecular Weigh	184.96	286.65
CAS No	144-48-9	51805-45-9

PROTOCOL

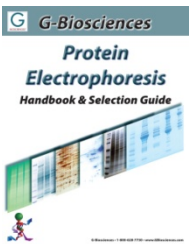
Protein reduction and alkylation may be performed in the same reaction tube, or IPG-Strips in two separate steps. We recommend reduction prior to alkylation as reducing agents added after iodoacetamide treatment will react with excess iodoacetamide.

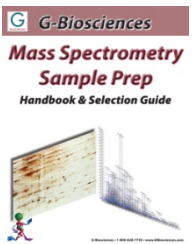
NOTE: *If a precipitate or crystal formation is seen in the Reductant or Alkylation Buffer, warm to room temperature and vortex to dissolve.*

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

Download our Protein Electrophoresis and Mass Spectrometry Sample Preparation Handbooks.





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