INSTRUCTIONS

Chloroacetamide, No-Weigh Format

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A39270

Number	Description
A39270	**Chloroacetamide, No-Weigh™ Format,** 10 × 2 mg
	Synonyms: 2-Chloroacetamide, CAA
	Molecular Weight: 93.51
	Formula: C_2H_4ClNO
	Storage: Upon receipt store product at 4°C.
	Note: Product labels have been provided for your convenience. Please label the vials using one of the labels provided in the Al foil pouch to avoid any confusion as you work with this No-Weigh reagent.

Introduction

Chloroacetamide is a sulfhydryl-reactive alkylating reagent used to block reduced cysteine residues for protein characterization and peptide mapping. Like iodoacetamide, chloroacetamide modification results in the covalent addition of a carbamidomethyl group (57.07 Da) to reduced cysteine sulfhydryls and prevents the formation of disulfide bonds.[1] However, chloroacetamide is less reactive and more stable in solution than iodoacetamide which results in more specific cysteine modification with less off-target labeling of other amino acids and side reactions.[2]

Traditional Sequential Reduction/Alkylation Procedure

Note: Chloroacetamide is mildly light-sensitive. Prepare solutions immediately before use and minimize exposure to light during alkylation. Although cysteine modification is the primary alkylation reaction, using too much chloroacetamide or incorrect buffer pH can result in alkylation of amines (lysine, N-termini), thioethers (methionine), imidazoles (histidine) and carboxylates (aspartate, glutamate). Dithiothreitol (DTT) can be used as an alternative reducing agent instead of TCEP for traditional reduction/alkylation but is not recommended for rapid "one-pot" reduction/alkylation.

1. Dissolve 5-100 µg of protein sample in 100 µL of 100 mM ammonium bicarbonate (pH 8.0) or 100 mM triethylammonium bicarbonate (pH 8.5) buffer.
2. Add 1 µL of 500 mM neutral Bond-Breaker™ TCEP Solution (Product No. 77720) to a final concentration of 5 mM TCEP and incubate sample at 37°C for 30 minutes.
3. Immediately before use, dissolve one tube of chloroacetamide (2 mg) with 107 µL of 100 mM ammonium bicarbonate (pH 8.0) or 100 mM triethylammonium bicarbonate (pH 8.5) buffer to make 200 mM chloroacetamide stock solution.
4. Add 11 µL of the 200 mM chloroacetamide to the sample for a final concentration of 20 mM chloroacetamide and incubate for 30 minutes at room temperature protected from light.
5. Perform proteolytic digestion before MS analysis or other application.

Rapid "One-Pot" Reduction/Alkylation Procedure

1. Dissolve 5-100 µg of protein sample in 100 µL of 100 mM ammonium bicarbonate (pH 8.0) or 100 mM triethylammonium bicarbonate (pH 8.5) buffer.
2. Add 1 µL of 500 mM neutral Bond-Breaker™ TCEP Solution (Product No. 77720) to a final concentration of 5 mM TCEP.
3. Immediately before use, dissolve one tube of chloroacetamide (2 mg) with 107 µL of 100 mM ammonium bicarbonate (pH 8.0) or 100 mM triethylammonium bicarbonate (pH 8.5) buffer to make 200 mM chloroacetamide stock solution.

4. Add 11 µL of the 200 mM chloroacetamide to the sample for a final concentration of 20 mM chloroacetamide and incubate for 10 minutes at 95°C protected from light.
5. Perform proteolytic digestion before MS analysis or other application.

Troubleshooting

Problem	Possible Cause	Solution
Sulfhydryls not blocked	Proteins not reduced	Reduce protein samples by adding DTT or TCEP before addition of chloroacetamide
	Chloroacetamide hydrolysis	Make chloroacetamide solutions immediately before each use and dispose of excess reconstituted reagent
Sulfhydryls partially blocked	Protein not denatured	Add 2% SDS or 8 M urea to protein sample to denature protein
	Insufficient chloroacetamide used	Use at least a 10-fold excess of chloroacetamide to sulfhydryls
	Incorrect reaction buffer	Avoid buffers that contain sulfhydryls or that are not at a slightly alkaline pH
	Insufficient reaction time or temperature	Allow reaction to proceed for 10 minutes at 95ºC or 30-60 minutes at room temperature
Amines or other functional groups labeled	Incorrect pH of the reaction buffer	Maintain the reaction buffer pH at 7.5-8.5
	Excessive chloroacetamide or incubation time	Reduce the amount of reagent or incubation time
		Quench excess reagent by addition of excess reducing agent after alkylation

Related Thermo Scientific Products

A39255 **DTT, No-Weigh™ Format,** 48 × 7.7 mg

A39271 **Iodoacetamide, No-Weigh™ Format,** 30 × 9.3 mg

A35349 **TCEP-HCl, No-Weigh Format,** 10 × 1mg

20490 **TCEP•HCl [Tris(2-carboxyethyl) phosphine hydrochloride],** 1 g

23011 **Methyl Methanethiosulfonate (MMTS),** 200 mg

23030 ***N*-Ethylmaleimide (NEM),** 25 g

77720 **Bond-Breaker™ TCEP Solution, Neutral pH,** 5 mL

77712 **Immobilized TCEP Disulfide Reducing Gel,** 5 mL

90055 **Trypsin Endoproteinase, modified, TPCK treated, MS Grade,** 5 × 20 µg

General References

1. Gurd, F.R.N. (1967). Carboxymethylation. *In* Methods in Enzymology, (C.H.W. Hirs, ed.), Vol. 11, p 532. Academic Press, New York.
2. Müller T. and Winter D. (2017). Systematic Evaluation of Protein Reduction and Alkylation Reveals Massive Unspecific Side Effects by Iodine-containing Reagents. *Mol Cell Proteomics* 16(7):1173-1187.