INSTRUCTIONS

Iodoacetamide, Single-Use

MAN0011661
Rev. B.0
Pub. Part No. 2162099

A39271

Number	Description
A39271	**Iodoacetamide, Single-Use,** 30 × 9.3 mg

Synonyms: 2-iodoacetamide, IAM

Molecular Weight: 184.96

Formula: C_2H_4INO

CAS: 144-48-9

Melting Point: 94°C

Appearance: White solid in amber, screw capped vials

Storage: Upon receipt store product at room temperature.

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

Iodoacetamide is a sulfhydryl-reactive alkylating reagent used to block reduced cysteine residues for protein characterization and peptide mapping. Alkylation with iodoacetamide after cystine reduction results in the covalent addition of a carbamidomethyl group (57.07 Da) and prevents the formation of disulfide bonds. Reducing agents added after alkylation will react with excess iodoacetamide.

Alkylation Procedure

Note: Iodoacetamide is unstable and light-sensitive. Prepare solutions immediately before use and perform alkylation in the dark. If iodoacetamide is present in limiting quantities and a slightly alkaline pH, cysteine modification will be the exclusive reaction. Excess iodoacetamide or non-buffered iodoacetamide reagent can also alkylate amines (lysine, N-termini), thioethers (methionine), imidazoles (histidine) and carboxylates (aspartate, glutamate).

1. Add 5 µl of 2% SDS and 45 µl of 200 mM ammonium bicarbonate (pH 8.0) to 20-100 µg of protein sample. Adjust volume to 100 µl with ultrapure water.
2. Add 5 µl of 200 mM Tris(2-carboxyethyl) phosphine hydrochloride (TCEP•HCl, Product No. 20490) and incubate sample at 55°C for 1 hour.
3. Immediately before use, dissolve one tube of iodoacetamide (9.3 mg) with 132 µl of 200 mM ammonium bicarbonate (pH 8.0) to make 375 mM iodoacetamide. Protect solution from light. **The maximum useable volume of the vial is 500µL.**
4. Add 5 µl of the 375 mM iodoacetamide to the sample and incubate for 30 minutes protected from light.
5. Proceed to proteolytic digestion before MS analysis or other processing.

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Troubleshooting

Problem	Possible Cause	Solution
Sulfhydryls not blocked	Iodoacetamide hydrolysis	Make iodoacetamide solutions immediately before each use and dispose of excess reconstituted reagent
Sulfhydryls partially blocked	Insufficient iodoacetamide used	Use at least a 10-fold excess of iodoacetamide to sulfhydryls
	Incorrect reaction buffer	Avoid buffers that contain sulfhydryls or that are not at a slightly alkaline pH
	Insufficient reaction time	Allow reaction to proceed for 30 minutes at room temperature
Amines or other functional groups labeled	Incorrect pH of the reaction buffer	Maintain the reaction buffer pH at 7.5-8.0
	Excess reagent or time	Reduce the amount of reagent or incubation time
		Acetone precipitate the alkylated sample to limit exposure during digestion

Related Thermo Scientific Products

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

20290 **DTT,** 5g

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

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

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

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

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

77701 **Immobilized Reductant Column**, 1 × 2 mL

90051 **Lys-C Endoproteinase, MS Grade,** 20 µg

90053 **Asp-N Endoproteinase, MS Grade,** 2 µg

90054 **Glu-C Endoproteinase, MS Grade,** 5 × 10 µg

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

90056 **Chymotrypsin Endoproteinase, TLCK treated, MS Grade,** 4 × 25 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. Vithayathil, P.J. and Richards, F.M. (1960). Modification of the methionine residue in the peptide component of ribonuclease S. *J Biol Chem* **235:**2343-51.
3. Cole, R.D, *et al.* (1958). On the cysteine content of hemoglobin. *J Biol Chem* **233:**1359-63.

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