

Pierce™ Iodoacetamide, Single-Use

Catalog Number A39271

Doc. Part No. 2162099 Pub. No. MAN0011661 Rev. C.0



WARNING! Read the Safety Data Sheets (SDSs) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves. Safety Data Sheets (SDSs) are available from thermofisher.com/support.

Product description

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. Pierce™ Iodoacetamide, Single-Use has the following properties:

- Synonyms: 2-iodoacetamide, IAA, IAM
- Molecular weight: 184.96
- Formula: C₂H₄INO
- CAS: 144-48-9
- Melting point: 94°C
- Appearance: White solid in amber, screw capped vials

Contents and storage

| Item | Cat. No. | Amount | Storage |
|-----------------------------------|------------------------|-------------|------------------|
| Pierce™ Iodoacetamide, Single-Use | A39271 | 30 x 9.3 mg | 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.

Alkylate sample

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, Cat. No. [20490](#)) and incubate the sample at 55°C for 1 hour.
3. Immediately before use, dissolve 1 tube of iodoacetamide (9.3 mg) with 132 µL of 200 mM ammonium bicarbonate (pH 8.0) to make 375 mM iodoacetamide. Protect the solution from light.

Note: The maximum useable volume of the vial is 500 µL.

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.

Troubleshooting

| Observation | Possible cause | Recommended action |
|---|--------------------------------------|---|
| 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. |

Limited product warranty

Life Technologies Corporation and/or its affiliate(s) warrant their products as set forth in the Life Technologies' General Terms and Conditions of Sale at www.thermofisher.com/us/en/home/global/terms-and-conditions.html. If you have any questions, please contact Life Technologies at www.thermofisher.com/support.

References

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Vithayathil PJ, Richards FM. (1960) Modification of the methionine residue in the peptide component of ribonuclease S. *J Biol Chem* 235:2343–2351.

Cole RD et al. (1958) On the cysteine content of hemoglobin. *J Biol Chem* 233:1359–1363.



Thermo Fisher Scientific | 3747 N. Meridian Road | Rockford, Illinois 61101 USA

For descriptions of symbols on product labels or product documents, go to thermofisher.com/symbols-definition.

Revision history: Pub. No. MAN0011661

| Revision | Date | Description |
|----------|-----------------|--|
| C.0 | 4 November 2022 | The format and content were updated. |
| B.0 | 23 July 2018 | The content was updated and new products were added. |
| A.0 | 17 October 2015 | New document for Pierce™ Iodoacetamide, Single-Use. |

The information in this guide is subject to change without notice.

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