INSTRUCTIONS

PIERCE
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IODO-BEADS® Iodination Reagent

28665 28666

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Number	Description
28665	**IODO-BEADS® Iodination Reagent**, 50 beads, contains sufficient material to perform 10-50 reactions using 0.1-0.5 mg of protein each
28666	**IODO-BEADS® Iodination Reagent**, 250 beads, contains sufficient material to perform 25-250 reactions using 0.1-0.5 mg of protein each
	Bead diameter: 3.175 mm
	Oxidative capacity: 0.55 ±0.05 µmol/bead
	Storage: Upon receipt store product desiccated at 4°C. Avoid exposing beads to moisture or reducing agents. Product is shipped at ambient temperature.

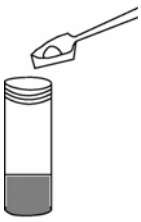
Introduction

IODO-BEADS® Iodination Reagent is *N*-chloro-benzenesulfonamide (sodium salt) immobilized on nonporous, polystyrene beads. This oxidizing agent was first reported by Markwell[1] as a means for iodinating proteins. IODO-BEADS® Iodination Reagent beads provide for effective iodination of peptides and proteins in 2-15 minutes across a broad pH range.

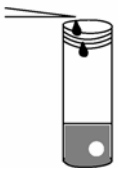
Radioactive iodine (^{131}I or ^{125}I) may be incorporated into protein either by enzymatic or chemical oxidation. In the chemical oxidation method, Na^{125}I or Na^{131}I is converted to its corresponding reactive radioactive iodine form. Historically, the oxidizing agent of choice was chloramine-T. However, because of its strong oxidizing properties, chloramine-T may damage proteins and thereby adversely affect their biological function for the intended experiment. IODO-BEADS® Iodination Reagent is milder than chloramine-T, generates sufficient radioactive iodine, and does not require a reduction step, which makes it advantageous for maintaining biological activity of proteins. Furthermore, IODO-BEADS® Iodination Reagent allows easy separation of the reagent from the reaction mixture, making it easy to control the reaction and limit direct contact of the oxidant with the protein.

Iodination with the IODO-BEADS® Iodination Reagent is compatible with many common buffer components. Detergents such as SDS, NP-40, and Triton® X-100, and denaturants such as urea, and high-salt concentrations (1.0 M NaCl) will not harm the reaction and may improve incorporation of the radioactive iodine by making tyrosines less hindered. Iodinations may be performed in phosphate, Tris, HEPES and other common buffers. The beads are not compatible with reducing agents.

Procedure Summary



1. Add beads to an ^{125}I-buffered solution in the reaction vial. Incubate for 5 minutes at room temperature.



2. Add protein in buffer to the reaction vial. Allow reaction to proceed for 2-15 minutes.

3. Separate reaction volume from the beads to terminate the iodination reaction.

Important Product Information

Beads and Protein: Use at least one bead for each 5-500 µg of tyrosine-containing peptide or protein dissolved in 0.1-1 ml of Reaction Buffer. Because beads can vary in oxidative capacity, use at least two beads per reaction for best results. The level of protein iodination can be controlled by varying the bead:protein ratio, the time of reaction, and amount of NaI added. If the protein or peptide does not contain tyrosine residues, which are the target of iodination, use SHPP or Sulfo-SHPP (see Related Pierce Products) to add these tyrosyl groups to the molecule.

Reaction Buffer: Phosphate-buffered saline (e.g., 0.1 M phosphate, 0.15 M NaCl; pH 7.2; Product No. 28372) or Tris, pH 5.5-7.5 (pH 6.5 is optimal). Phosphate buffered Saline (PBS) results in higher ^{125}I incorporation than Tris. Other buffers may be used provided they are free of reducing agents and anti-oxidants. Avoid organic solvents, such as DMSO and DMF, that readily dissolve polystyrene.

Temperature and time for iodination: Room temperature is optimal for iodine labeling. However, the reaction may also be performed at 4°C, but with reduced efficiency. Typical reaction time is 2-15 minutes. The optimal reaction time for each protein must be determined empirically.

Example Procedure for Protein Iodination

A. Additional Materials Required

- Reaction Buffer: see description in Important Product Information above
- Sodium iodide ($Na^{125}I$), carrier-free
- Microcentrifuge tube or glass reaction vial, such as Reacti-Vial™ Small Reaction Vial (Product No. 13222 or 13223)
- Desalting column or dialysis unit to separate labeled protein/peptide from excess unincorporated iodine (see Step 5)

B. Procedure

1. Immediately before use, wash beads with 500 µl of Reaction Buffer per bead. Dry the beads on filter paper. (This wash step removes any loose particles and reagent.)
2. Add beads to a solution of carrier-free $Na^{125}I$ (approximately 1 mCi per 100 µg of protein) diluted with Reaction Buffer and incubate for 5 minutes. A small reaction vial is optimal for this step.
3. Dissolve or dilute protein in Reaction Buffer and add to the reaction vessel. Incubate reaction mixture for 2-15 minutes.

   Note: For best results, perform a time study (see Time Study Procedure below) to determine optimal reaction time.
4. Stop the reaction by removing the solution from the reaction vessel. Once beads are separated from the solution, the reaction will stop.

   Note: Beads may be washed with a small quantity of Reaction Buffer to recover any protein from the bead surface.
5. Zeba™ Desalt Spin Columns (e.g., Product No. 89891) may be used to remove excess $Na^{125}I$ or unincorporated ^{125}I from the iodinated protein (if the protein is larger than 7 kDa). Alternatively, use a Slide-A-Lyzer® Dialysis Cassette (e.g., Product No. 66382) and dialyze the iodinated protein against a buffer suitable for the specific downstream application. For peptides larger than 2 kDa, use Slide-A-Lyzer® Dialysis Cassette, 2K MWCO (see Related Pierce Products).

Example Time Study Procedure

This is a summary of the method described by Cheng and Rudick.[2]

1. Remove 5 µl of the reaction mixture every 30 seconds, removing the first aliquot when the bead is added (time zero).
2. Apply each aliquot to separate squares of nitrocellulose. Support the squares using a non-adsorbent surface.
3. Place squares in a vials and count for gamma radiation. The total counts will be approximately the same for each square.
4. Prepare Tris-glycine/NaI buffer by titrating 25 mM Tris base with 192 mM glycine to pH 8.3, add methanol to 20% and sodium iodide to a final concentration of 10 mM.
5. To remove free ^{125}I, wash squares 4 × 5 minutes with gentle agitation with 50 ml of Tris-glycine/NaI buffer.
6. Count gamma radiation on the washed squares. Subtract the zero time counts of washed squares from the other counts to determine net radioactivity for each incubation-time aliquot. This provides the radioactivity incorporated into the protein.
7. The efficiency of radioiodine incorporation may be measured by dividing the net radioactivity of the washed membranes by the radioactivity determined from the unwashed membranes after sample application.

Example Procedure for Cell-Surface Iodination

A. Additional Materials Required

- Phosphate-buffered saline (PBS): 0.1 M phosphate, 0.15 M NaCl; pH 7.2 (Product No. 28372)
- $Na^{125}I$
- Carrier iodide: 25 mM NaI in PBS (optional)

B. Procedure

1. Wash 10^5-10^6 cells 3 times with PBS.
2. Prepare IODO-BEADS® Iodinating Reagent by washing the beads twice with 0.5 ml PBS per bead. Dry the beads on filter paper. (This wash step removes any loose particles and reagent.)

   Note: For best results, use six beads per 10^5-10^6 cells. Specific activity may be controlled by changing the number of beads. Using more than six beads per 10^5-10^6 cells will not increase specific activity and may reduce protein recovery.
3. Resuspend cells in 4 ml PBS and add ^{125}I (approximately 1 mCi per 100 µg wet cells), 40 µl carrier iodide (optional) and the iodinating beads.

   Note: For best results, determine the optimal amount of carrier iodide to add for the specific application.
4. Incubate the samples at room temperature for 2-15 minutes.

   Note: For best results, perform a time study to determine optimal reaction time. Iodination may be performed at 4°C; however, the efficiency will be reduced.
5. Stop the reaction by removing the iodinating beads with forceps.
6. Wash cells 3 times with PBS, to remove non-reacted ^{125}I. Cell-surface proteins may be solubilized and analyzed by SDS-PAGE.

Troubleshooting

Problem	Cause	Solution
Iodination of protein is low	Absence of tyrosines available for labeling	Use Bolton-Hunter Reagent (Product No. 27710) or its water-soluble analog Sulfo-SHPP (Product No. 27712) to modify primary amines on the protein with tyrosine-"like" functional groups **Note:** These reagents may be iodinated first and the protein subsequently reacted with the iodination reagent
	Insufficient surface tyrosines available for labeling	Use high-salt (1.0 M NaCl), detergents or denaturants to allow better access to "buried" tyrosines
Protein could not be iodinated	Reducing agents or anti-oxidants are interfering with labeling	Avoid 2-mercaptoethanol, dithiothreitol, sodium borohydride, sodium cyanoborohydride, glycerol or any other compound with reducing properties
Protein is being degraded	The protein is susceptible to oxidation	Allow the bead to oxidize the $Na^{125}I$ solution as a separate step from the protein iodination and then transfer the oxidized iodine to the protein solution **Note:** Iodine incorporation is much less efficient than the standard method but may be necessary for easily oxidized proteins
		Perform a time study to determine the optimal reaction time

Appendix

IODO-BEADS® Iodination Reagent is susceptible to inactivation from exposure to reducing agents or moisture. To verify if the beads have sufficient activity, use this simple qualitative test, which was first described by Lee and Griffiths.[6]

1. Dissolve 125 µg of potassium iodide in 100 µl of 0.1 M sodium phosphate buffer, pH 7.0.
2. Add one unused bead to this solution.

An active bead will turn yellow to brown in less than 15 seconds and the solution will turn yellow a few seconds later. A bead with low activity will require several minutes or longer to produce the color change.

Related Pierce Products

89889	**Zeba™ Desalt Spin Columns, 2 ml**, 5 columns, for desalting 200-700 µl samples
89891	**Zeba™ Desalt Spin Columns, 5 ml**, 5 columns, for desalting 500-2,000 µl samples
66383	**Slide-A-Lyzer® Dialysis Cassettes* (10K MWCO), 0.1-0.5 ml,** 10 cassettes
66380	**Slide-A-Lyzer® Dialysis Cassettes* (10K MWCO), 0.5-3 ml,** 10 cassettes
66205	**Slide-A-Lyzer® Dialysis Cassettes* (2K MWCO), 0.2-0.5 ml,** 10 cassettes
66203	**Slide-A-Lyzer® Dialysis Cassettes* (2K MWCO), 0.5-3 ml,** 10 cassettes
27710	**Bolton-Hunter Reagent (SHPP),** 1 g
27712	**Water-Soluble Bolton-Hunter Reagent (Sulfo-SHPP),** 100 mg
28601	**IODO-GEN® Pre-Coated Iodination Tubes,** 10/pkg
28600	**IODO-GEN® Iodination Reagent,** 1 g

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2. Cheng, Hui and Rudick, M.J. (1991). A membrane blotting method for following the time course of protein radioiodination background references using IODO-BEADS. *Anal. Biochem*. **198:** 191-193.
3. Lee, D.S.C. and Griffiths, B.W. (1984). Comparative studies of IODO-BEADS® and chloramine-T methods for the radioiodination of human alpha-fetoprotein. *J. Immunol. Meth.* **74(**181).

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Current versions of product instructions are available at www.piercenet.com. For a faxed copy, call 800-874-3723 or contact your local distributor.