

Pierce[®] Iodination Beads

28665 28666

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Number	Description
28665	Pierce Iodination Beads , 50 beads, contains sufficient material to perform 10 to 50 reactions using 0.1 to 0.5mg of protein each
28666	Pierce Iodination Beads , 250 beads, contains sufficient material to perform 25 to 250 reactions using 0.1 to 0.5mg of protein each Bead diameter: 3.175mm Oxidative capacity: 0.55 ± 0.05µmol/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

The Thermo Scientific Pierce Iodination Beads (previously called “IODO-BEADS”) consist of the iodination reagent *N*-chloro-benzenesulfonamide (sodium salt) immobilized on nonporous, polystyrene beads. This oxidizing agent was first reported by Markwell¹ as a means for iodinating proteins. Iodination Beads provide for effective iodination of peptides and proteins in 2-15 minutes across a broad pH range.

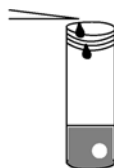
Radioactive iodine (¹³¹I or ¹²⁵I) can be incorporated into protein either by enzymatic or chemical oxidation. In the chemical oxidation method, Na¹²⁵I or Na¹³¹I is converted to its corresponding reactive radioactive iodine form. Historically, the oxidizing agent of choice was chloramine-T. However, because of its strong oxidizing properties, chloramine-T may damage proteins and thereby adversely affect their biological function for the intended experiment. Iodination with these beads 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, Iodination Beads allow for quick and complete separation of the reagent from the reaction mixture, making it easy to control the reaction time and to limit direct contact of the oxidant with the protein.

Iodination with Pierce Iodination Beads 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 ¹²⁵I-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 μ g of tyrosine-containing peptide or protein dissolved in 0.1-1mL 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 Thermo Scientific Products Section) to add these tyrosyl groups to the molecule.

Reaction Buffer: Phosphate-buffered saline (e.g., 0.1M phosphate, 0.15M 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, which 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
- 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 μ L 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 μ g 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. Thermo Scientific Zeba Spin Desalting 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 7kDa). Alternatively, use a Thermo Scientific 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 2kDa, use Slide-A-Lyzer[®] Dialysis Cassette with a 2K MWCO (see web site for complete listing).

Example Time Study Procedure

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

1. Remove 5 μ L 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 25mM Tris base with 192mM glycine to pH 8.3, add methanol to 20% and sodium iodide to a final concentration of 10mM.
5. To remove free ^{125}I , wash squares 4 \times 5 minutes with gentle agitation with 50mL 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): 0.1M phosphate, 0.15M NaCl; pH 7.2 (Product No. 28372)
- Na¹²⁵I
- Carrier iodide: 25mM NaI in PBS (optional)

B. Procedure

1. Wash 10⁵-10⁶ cells 3 times with PBS.
2. Prepare Iodination Beads by washing them twice with 0.5mL 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 10⁵-10⁶ cells. Specific activity may be controlled by changing the number of beads. Using more than six beads per 10⁵-10⁶ cells will not increase specific activity and may reduce protein recovery.

3. Resuspend cells in 4mL PBS and add ¹²⁵I (approximately 1mCi per 100μg wet cells), 40μL 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 ¹²⁵I. 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 (1.0M 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

Pierce Iodination Beads are 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.³

1. Dissolve 125µg of potassium iodide in 100µL of 0.1M 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 Thermo Scientific Products

27710	Bolton-Hunter Reagent (SHPP), 1g
27712	Water-Soluble Bolton-Hunter Reagent (Sulfo-SHPP), 100mg
28601	Pierce Iodination Tubes, 10/pkg, glass test tubes coated with Pierce Iodination Reagent
28600	Pierce Iodination Reagent, 1g

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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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