INSTRUCTIONS

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Pierce Pre-Coated Iodination Tubes

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Number	Description
28601	**Pierce Pre-Coated Iodination Tubes,** 10 borosilicate test tubes (12 × 75mm) coated with 50µg Pierce Iodination Reagent (formerly called "IODO-GEN" Iodination Reagent) Tube-to-tube variation: ≤ 5% **Storage:** Upon receipt store at room temperature protected from moisture. After opening barrier pouch, close the interior resealable bag containing the tubes with the yellow closures and desiccant.

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Introduction

The Thermo Scientific™ Pierce™ Pre-Coated Iodination Tubes are a ready-to-use source of Pierce Iodination Reagent that provides convenient, consistent radioiodination of proteins and cell membranes.[1] Pierce Iodination Reagent (1,3,4,6-tetrachloro-3α, 6α-diphenylglycouril; formerly called "IODO-GEN" Iodination Reagent) is an effective and mild oxidation reagent. This reagent is as effective as enzymatic methods for iodination of externally exposed residues and as effective as chloramine-T for general protein iodination, in addition to being more mild[1-5] resulting in limited oxidative damage and retained cell viability.[2]

During oxidative reactions in the Pre-Coated Tubes, which are coated at the bottom with about 100µL, the iodination reagent remains adherent to the vessel because it is insoluble in typical aqueous media. This feature enables the sample to be decanted from the coated vessel to terminate the oxidative reaction. Although Pierce Iodination Reagent can be coated to any borosilicate glass surface, coating and drying procedure is very difficult to achieve in the typical laboratory without the reagent becoming inactive or flaking from the surface. The ability of the reagent to adhere tightly to a vessel depends on temperature, humidity, solvent and evaporation rate, and the coating procedure can be time-consuming, troublesome and difficult to reproduce. We have optimized these conditions in preparing the Pre-Coated Iodination Tubes, providing a ready-to-use product for convenient iodination of typical protein and peptide samples.

Pierce Pre-Coated Iodination Tubes eliminate the troublesome coating step and offers a tightly adherent film of iodination reagent that will not flake off the tube walls. Each tube is consistently coated and exhibits less than 5% variation from tube-to-tube. The consistent coating results in highly reproducible radioiodinations.

Important Product Information

- Exercise caution when working with radioactive iodide. Only appropriately trained personnel are to perform radioiodination procedures.
- The efficiency of iodination is independent of pH values between 6 and 8.5. Efficiency decreases by ~15% at pH 5.0.[6] Pierce Iodination Reagent is virtually insoluble in aqueous media at pH ≤ 8.5. Extremely alkaline solutions will increase the solubility of the reagent.
- Removal of the iodide solution from the Pre-Coated Test Tube terminates the oxidative production of the active iodinating species. After removal, the active iodinating species decays to molecular iodine within 15 minutes at room temperature.[6,7] After iodine incorporation, it is common to spike the iodinated sample with a carrier protein before gel filtration to remove excess radioactive iodine. To prevent iodination of the carrier protein, quench the solution containing oxidized iodide for active iodous species by adding a reducing agent or iodination scavenger, such as tyrosine, *p*-hydroxyphenyl propionic acid or *p*-hydroxyphenyl acetic acid, or allow it to decay to molecular iodine.
- When proteins or peptides contain few available tyrosines for iodination, Sulfo-SHPP (Product No. 27712) is a useful reagent for introducing iodinatable groups onto lysine side chains or onto other primary amine-containing molecules.
- Iodous ions (I^+) are produced by oxidation of iodide (I^-) by Pierce Iodination Reagent and undergo electrophilic attack at the ortho ring position of tyrosine at neutral pH values.[4,8] At pH ≥ 9, the imidazole ring of histidine can be labeled.[4] Mono- and di-iodinated derivatives of the tyrosine phenolic ring can be produced. The cytosine of DNA and RNA has also been radiolabeled via the use of Pierce Iodination Reagent.[9]

Example Protocol I: The Chizzonite Indirect Method for Iodination

Dr. Chizzonite used Pierce Pre-Coated Iodination Tubes (formerly called "IODO-GEN") for indirect labeling of proteins and peptides. The Chizzonite method is the preferred and standard method for radiolabeling proteins and peptides. This method allows the sample to never contact the iodination reagent, which avoids the potential for oxidative damage to labile material as well as the potential for protein loss by aggregation or hydrophobic interaction with the coating. Furthermore, preactivating the radioactive iodide allows flexibility in the choice of reaction vessel to use. The preactivation protocol facilitates scaling up or down as needed.

A. Materials Required

Tris Iodination Buffer	25mM Tris•HCl, pH 7.5, 0.4M NaCl. This buffer is used for iodide oxidation and for radioiodination of protein. Store buffer at 4°C.
Protein Stock Solution	Prepare 0.3-1.0nmol of protein in 100µL of Tris Iodination Buffer in a 1.5mL screw cap tube.
High Tris Buffer (Optional)	0.125M Tris•HCl, pH 6.8, 0.15M NaCl. This buffer can be used in place of the standard Tris Buffer where higher buffering capacity is needed for more alkaline solutions of $Na^{125}I$.
Tris/BSA Buffer	0.25% bovine serum albumin (2.5mg/mL), 25mM Tris•HCl, pH 7.5, 0.4M NaCl, 5mM EDTA, 0.05% sodium azide. This buffer is used for blocking nonspecific binding sites on the desalting column, as a diluent for the radiolabeled protein, and as the wash buffer during desalting.
Tris/NaCl/EDTA Buffer	25mM Tris•HCl, pH 7.5, 0.4M NaCl, 5mM EDTA, 0.05% sodium azide. This buffer can be used as a substitute for the Tris/BSA buffer for protein dilution and as the wash buffer during desalting when the inclusion of BSA as a carrier protein is not desired.
Scavenging Buffer	10mg tyrosine/mL in Tris Iodination Buffer or PBS, pH 7.4. This solution is saturating and crystals do not completely dissolve; *p*-hydroxyphenylpropionic acid or *p*-hydroxyphenylacetic acid can be used as substitutes for tyrosine.
Desalting Column	10mL bed volume (e.g., Polyacrylamide Desalting Columns, 6K MWCO, Product No. 43243)

B. Procedure

1. Wet a Pierce Pre-Coated Iodination Tube with 1mL of Tris Iodination Buffer and decant.
2. Add 100µL of Tris Buffer to the pre-coated tube. Add buffer directly to the bottom of the tube; do not allow buffer to drain down the tube wall.
3. Add 10µL (1.0mCi) of $Na^{125}I$ (e.g., from MP Biomedicals or other supplier) and mix.

   Note: The amount of $Na^{125}I$ may be increased to 20µL for labeling larger amounts of protein or to increase the radiospecific activity of the iodinated protein.
4. Allow iodide to activate for 6 minutes at room temperature. Swirl the tube every 30 seconds.
5. Remove and add the activated iodide to the protein solution and mix.
6. React for 6-9 minutes at room temperature. Mix by gently flicking the tube every 30 seconds.
7. Add 50µL of Scavenging Buffer. Mix and incubate for 5 minutes with additional flicking at 1 and 4 minutes.
8. Add 1.0mL of Tris/BSA Buffer and mix. Remove a 5µL aliquot for determination of total ^{125}I incorporation. (If BSA is omitted from the reaction, use 1.0mL of Tris/NaCl/EDTA Buffer.)
9. Add sample to a 10mL bed volume desalting column that has been equilibrated with 20mL of Tris/BSA Buffer.

   Note: If the protocol is being modified to omit BSA as a carrier protein, after column equilibration with Tris/BSA Buffer, add 20mL of Tris/NaCl/EDTA Buffer.
10. Wash tube containing the iodinated protein with 0.5mL of Tris/BSA Buffer and add this to the column to increase recovery of the iodinated protein. Use 0.5mL of Tris/NaCl/EDTA Buffer if BSA is omitted.
11. Recover sample by adding 1.0mL aliquots of Tris/BSA Buffer. Collect 6 × 1mL fractions in 12 × 75mm polypropylene tubes. If BSA is omitted, use 0.5mL of Tris/NaCl/EDTA Buffer. Scan eluted fractions for radioactivity.
12. After the last fraction has emerged from the column, seal the tip, recap column and dispose column as radioactive waste.
13. Determine protein bound and free ^{125}I by standard TCA precipitation procedures.
14. Determine radiospecific activity of the labeled protein. Typically, the radiospecific activity will be 2500-4000cpm/fmole when 0.3nmole of protein is labeled by this procedure. The radiospecific activity can be manipulated to vary from 500-7500cpm/fmol by using higher or lower amounts of protein or ^{125}I-Na in the protocol.
15. Dilute the labeled protein with Tris/BSA buffer to an activity of $1\text{-}2 \times 10^8$ cpm/mL and store at 4°C. Labeled antibodies are usually stable for up to eight weeks; very labile proteins may retain stability for 3-4 weeks.

Example Protocol II: Direct Method for Iodination

The following protocol is based on the method by Mary Ann K. Markwell.[2] Additional notes and suggestions are provided to address commonly asked questions and variations. In this method, the material to be iodinated is added directly to the Pierce Pre-Coated Iodination Tube (formerly called "IODO-GEN" Pre-Coated Iodination Tube).

1. Dissolve the sample in an appropriate buffer and add to a pre-coated tube.

   Note: Choose a buffer and temperature that is compatible with the specific sample and biological system. The optimal temperature and pH range is 0-37°C and 6-8.5, respectively. For cells a medium, such as Dulbecco's PBS plus glucose, may be used to maintain cells during iodination. Avoid using media containing tyrosine or serum, as most sera contain appreciable amounts of NaI. Also avoid 2-mercaptoethanol, dithiothreitol and cysteine.
2. Add carrier-free $Na^{125}I$ to the solution in the pre-coated tube. Typically, 500µCi of $Na^{125}I$ is used per 100µg of protein.

   Note: Carrier NaI or KI is sometimes used to drive the reaction, to label inner membrane proteins, or for safety reasons. However, some of the iodine incorporated will not be radioactive. Carrier iodide from 0.25mM to 1mM may be used with 0.5-1.0mCi of radioiodide. If a "hot" label is required, use more carrier-free radioiodide or omit the non-radioactive carrier iodide.
3. Allow the reaction to proceed for 10-15 minutes. If the sample is stable and a higher specific radioactivity is desired, the reaction time may be increased. Agitate the vessel for best results. Perform a time study to optimize the procedure. Shorter reaction times may be necessary if the sample is labile.

4. Remove sample from the reaction vessel to terminate the iodide oxidation.
5. If no carrier iodide has been used in step 2, to enhance safety add NaI or KI to a final concentration of 1mM to the reaction mixture.

   Note: If remaining non-reacted ^{125}I and small amounts of the active iodinating species interferes with subsequent experimental steps, tyrosine or other phenolic molecules, such as *p*-hydroxyphenylpropionic acid or *p*-hydroxyphenyl-acetic acid may be added to the mixture as iodination scavengers. Alternatively, reducing agents, such as sodium thiosulfate or sodium bisulfite, may be added at 1µmol. Excess iodide can be removed by gel filtration. Use a desalting column, such as Dextran Desalting Column (Product No. 43230), to separate ^{125}I and scavenged ^{125}I from the iodinated protein.

Example Protocol III: Iodination of Crosslinkers

The iodination of crosslinkers is performed rapidly, which limits hydrolysis of the NHS-ester portion of the crosslinker. Perform the following steps in a darkened room if working with a photoreactive phenyl azide crosslinker, such as SASD.

A. Materials Required

- Iodinatable crosslinker
- Phosphate Buffered Saline (PBS): 0.1 M sodium phosphate, 0.15 M NaCl; pH 7.2 (e.g., BupH™ Phosphate Buffered Saline Pack, Product No. 28372).
- Dimethylsulfoxide (DMSO)
- Pierce Pre-Coated Tube

B. Procedure

1. Add 90µL of PBS to a Pierce Pre-Coated Iodination Tube.
2. Dissolve 5.5µmol of crosslinker in 50µL of dry DMSO.
3. Make a 1:20 dilution of the stock solution by adding 19µL of PBS to 1.0µL of stock solution and mix well. Immediately remove 10µL and add it to the tube containing the PBS and mix well (this solution will contain 55nmol of crosslinker). This crosslinker working solution is not stable, therefore, proceed immediately to step 4.
4. Add 40µCi $Na^{125}I$ and 18.5nmol potassium iodide (KI) in 10µL of 0.1M sodium phosphate, pH 7.4.

   Note: KI is optional in this reaction and is used to increase the efficiency of iodine incorporation; however, including cold KI will decrease the specific activity of the crosslinker.
5. Allow reaction to proceed for 30 seconds. Stop the reaction by removing the solution from the tube.

   Note: Most applications will benefit from adding an iodine scavenger to prevent potential tyrosine iodination on the protein to be reacted with the crosslinker caused by excess reactive iodine. Scavengers such as 4-hydroxyphenylacetic (or propionic) acid may be added (20nmol) for this purpose; do not use tyrosine nor reducing agents as they will interfere with the NHS-ester reaction.
6. Immediately add the iodinated crosslinker into a tube containing 16nmol of protein in 300µL of borate buffer and allow to react for 30 minutes. Excess scavenger and iodide can be removed from the iodinated protein by gel filtration. Depending on the downstream application, a separation step may not be required.

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