INSTRUCTIONS

Thermo SCIENTIFIC

Pierce Iodination Reagent

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28600

Number	Description
28600	**Pierce Iodination Reagent,** 1g Chemical Name: 1,3,4,6-tetrachloro-3α-6α-diphenylglycouril Molecular Weight: 432.09 CAS#: 51592-06-4 **Storage:** Upon receipt store refrigerated and desiccated. Product shipped at ambient temperature.

Introduction

The Thermo Scientific™ Pierce™ Iodination Reagent (1,3,4,6-tetrachloro-3α-6α-diphenylglycouril) was first described by Fraker and Speck as a reagent for the iodination of tyrosyl groups in proteins and cell membranes. The reagent (previously called "IODO-GEN") is insoluble in aqueous media and can be coated onto a reaction vessel for use in solid-phase procedures.[1] Reaction vessels can be prepared in advance and stored indefinitely in a desiccator over a drying agent until needed. Pierce Iodination Reagent provides a reproducible, gentle and technically simple means for radioiodination of proteins and peptides. It is as effective as enzymatic iodination methods for exposed residues and as effective as chloramine-T iodination methods in general.[2,3]

Pierce Iodination Reagent can be used to iodinate phenolic groups on crosslinkers or other protein modification reagents before or after a protein, peptide, or cell surface is reacted with the modification reagent. Some proteins and peptides contain few or no tyrosines as sites for iodination. Certain other molecules are inactivated when their tyrosines are iodinated. The water-soluble analog, Sulfo-SHPP (Prod. No. 27712), is a useful reagent for introducing iodinatable tyrosine-like residues to primary amine-containing molecules.

By optimizing the reaction buffer conditions, iodination of intact cells can be limited to cell surface or directed more toward inner membrane proteins. Inclusion of a small excess of carrier iodide, using high salt concentrations or employing detergents can be used to improve inner membrane protein labeling. Pierce Iodination Reagent is unaffected by denaturing conditions or the presence of enzymatic inhibitors in the reaction mixture. Because no additional steps are needed for terminating the reaction, samples with volumes less than 100µL can be easily accommodated. Pierce Iodination Reagent is also useful as a solid-phase reagent for the oxidative removal of sulfhydryl groups that interfere with the Lowry protein assay.[4]

Standard Protocol for Radioiodination of Proteins and Peptides

A. Prepare the Reaction Vessel

Note: Failure to properly plate the reagent on the reaction vessel may cause the oxidizing agent to remain with the protein solution, resulting in continued exposure of the protein to oxidation.

1. Choose a glass reaction vessel appropriate for the volume of sample to be treated. Ensure that the vessel is clean and dry.

Table 1. Suggested reaction vessels for various reaction volumes.

Volume	Reaction Vessel
10-100µL	12 × 75mm test tube (see Related Products)
100-1000µL	20 × 150mm test tube
1-10mL	20mL scintillation vial
10cm culture dish	Two 22 × 22mm coverslips

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2. Add the desired amount of the Iodination Reagent to a small volume of dry organic solvent, such as chloroform or methylene chloride. Dissolve 10-100µg of Iodination Reagent per 100-500µL of solvent. Plan to use ≤ 10µg of Iodination Reagent per 100µg of protein or polypeptide, or 100µg of Reagent per 1×10^7 cells.

   Note: Do not use dimethyl sulfoxide (DMSO) as the solvent as it is not compatible with the Iodination Reagent. Because Pierce Iodination Reagent remains in the solid phase during the reaction, it is important that the reagent be evenly plated on the reaction vessel.

3. Add the Iodination Reagent solution to the clean, dry glass reaction vessel. Slowly evaporate off the solvent using dry nitrogen or other dry inert gas. Slow evaporation is important. If the solvent is removed too quickly, the solid will precipitate in clumps or flecks instead of forming a fine, hard-to-see film.

   Note: We do not recommend leaving vials to dry in a hood without nitrogen. A Pasteur pipette or evaporating manifold works well for applying the stream of nitrogen. Adjust the flow gas so that it can barely be felt on your skin. For best results, the needle or pipette tip should be placed so that it is just above or below the lip of a vial and keep the reaction vessel stationary during the evaporation.

4. After the solvent is evaporated, use the reaction vessel immediately or seal and store it desiccated until use.

B. Radioiodination Procedure

1. Dissolve the sample in an appropriate buffer. Choose a buffer and temperature that is compatible with your sample and biological system. Use 0-37°C and a buffer at pH 4.4-9.0. Borate, phosphate, Tris, HEPES, normal saline, and Krebs-Ringer solution are all compatible. Avoid high concentrations of detergents or organic solvents that could remove the iodination reagent from the glass surface. Also avoid reducing agents and antioxidants, 2-mercaptoethanol, dithiothreitol, cysteine, glycerol.

   Note: To iodinate monolayers of cells, plated coverslips with the Iodination Reagent and float them on a thin layer of medium over the monolayer. To maintain the cells during the iodination, use Dulbecco's PBS plus glucose. Avoid using media containing tyrosine or serum. Most sera also contain appreciable amounts of NaI.

2. Rinse the plated reaction vessel with sample buffer to remove loose microscopic flakes of iodination reagent. If visible flakes are present, recoating is necessary.

3. Add carrier-free $Na^{125}I$ to the reaction vessel solution. Use 500µCi of $Na^{125}I$ per 100µg protein or 10cm culture dish.

   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.

4. Allow the reaction to proceed from 10-15 minutes with agitation. Increase the time if the sample is stable and a higher level of radioactivity is desired. Perform a time-course experiment to optimize the procedure. Shorter reaction times may be necessary if the sample is degraded easily by the oxidation or if the sample is unstable in the aqueous sample buffer.

5. Remove sample from the reaction vessel to terminate the labeling reaction. Addition of a reducing agent is not necessary.

6. To ensure safe handling, add NaI or KI to a final concentration of 1mM to the reaction mixture if no carrier iodide was used in step 3 and if not proceeding to another labeling step (as when using a crosslinker).

7. Some non-reacted ^{125}I might remain, including a small amount of the active species. If this will interfere with the intended experiment, remove it by dialysis, centrifugation or gel filtration methods (e.g., Thermo Scientific™ Zeba™ Spin Desalting Columns) to separate ^{125}I from the iodinated protein. Tyrosine (sodium salt) or tyrosine-like molecules such as 4-hydroxyphenyl propionic acid or 4-hydroxyphenyl acetic acid can be added to the reaction mixture to "take up" active radioiodide if proceeding without a gel filtration or dialysis step.

Reaction Condition Modifications

By modifying reaction conditions, one can iodinate residues normally buried within the protein or membrane structure. In the first modification, increase the carrier NaI concentration to 1mM. A yellow color characteristic of molecular iodine is generated during the course of the reaction. This modification has proven to be particularly useful for iodinating lipoproteins, the interior proteins of viruses and the cytosol of eukaryotic cells while maintaining structural integrity.

If structures are disrupted before iodination by the addition of detergents, such as Triton™ X-100 or sodium dodecyl sulfate, and/or with chaotropic reagents such as urea, the specific radioactivity achieved in protein labeling is equal to or greater than that achieved with chloramine-T. Samples solubilized in SDS prior to iodination can be used immediately for SDS-PAGE without further preparation other than the addition of carrier NaI. During electrophoresis, use appropriate shielding for gamma emitters. Any unreacted ^{125}I will appear in the lower reservoir buffer (at the cathode).

Iodination of a Crosslinker or Labeling Reagent and Subsequent Reaction with a Protein

Perform the following steps in a darkened room if working with a photoreactive phenyl azide crosslinker. Perform steps 5-7 quickly to limit hydrolysis of NHS-Ester reactive groups. If labeling a molecule with a phenolic group that does not also have an NHS-Ester group, then the timing is not usually as critical unless the molecule is unstable in reaction solutions.

1. Dissolve 1mg of Pierce Iodination Reagent in 100µL of chloroform.
2. Pipette this solution into a small glass vial.
3. Evaporate the chloroform under nitrogen (takes approx. 10 minutes). Cap the container and store frozen and desiccated.
4. Dissolve 5.5µmol of an NHS-Ester crosslinker in 50µL of dry DMSO in a separate vial.
5. Make a 1:20 dilution of the crosslinker stock solution by adding 190µL of phosphate buffer per 10µL of the dissolved crosslinker. Mix well and immediately remove 10µL of this diluted stock solution and add it to a new vial containing 90µL of phosphate buffer (this solution now contains 55nmol of NHS-Ester). Mix well again and immediately proceed to the next step. (This is the NHS working solution and it is not stable.)
6. Add working solution from step 5 to the vessel coated with Iodination Reagent (prepared in steps 1-3). Add 40µCi Na^{125}I and 18.5nmol potassium iodide (KI) in 10µL of 0.1M sodium phosphate, pH 7.4.

   Note: It is not necessary to add KI in this reaction. It is used to accelerate the incorporation of iodine; however, some of the iodine incorporated will not be radioactive.
7. Allow the reaction to proceed for exactly 30 seconds. Stop the reaction by removing the solution from the container.

   Note: For some applications it may be necessary to add an excess of an iodide scavenger (> 20nmol) such as tyrosine (sodium slat), 4-hydroxyphenylacetic (or propionic) acid at this point to prevent incorporation of iodine into tyrosines or histidine residues of the protein to be modified.
8. Immediately transfer iodinated crosslinker to protein sample to initiate crosslinking reaction. Pipette the solution from step 7 into a tube containing 16nmol of protein in 300µL of borate buffer and incubate for 30 minutes. Separate excess crosslinker and iodine from protein(s) by gel filtration or dialysis. Solvent extraction may also be used to separate reactants, product and by-products.

Related Thermo Scientific Products

Other Iodination Reagent Products:

28601	**Pierce Iodination Tubes,** 10/pkg, glass test tubes coated with Pierce Iodination Reagent
28665, 28666	**Pierce Iodination Beads,** 50 or 250 polystyrene beads coated with iodination reagent

Tyrosyl-Addition Reagents:

27712	**Water-Soluble Bolton-Hunter Reagent (Sulfo-SHPP),** 100mg

References

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