INSTRUCTIONS

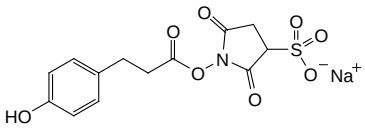
Water-Soluble Bolton-Hunter Reagent

27712

0476.3

Number	Description
27712	**Water-Soluble Bolton-Hunter Reagent (Sulfo-SHPP),** 100mg (sulfosuccinimidyl-3-[4-hydroxyphenyl]propionate)

Molecular Weight: 365.29



Storage: Upon receipt store at -20°C. Product is shipped at ambient temperature.

Introduction

The Thermo Scientific™ Water-Soluble Bolton-Hunter Reagent (Sulfo-SHPP) conjugates tyrosine-like residues to primary amines for increasing the yield of subsequent iodination. The reagent is ideal for iodinating proteins that do not contain tyrosine residues or contain tyrosine residues with limited accessibility to iodination. Labeling with Sulfo-SHPP also can help to preserve tyrosine residues that are essential for protein function or immunogenicity.

Water-Soluble Bolton-Hunter Reagent contains an *N*-hydroxysuccinimide (NHS) ester reactive group. NHS esters react efficiently with primary amino groups (-NH_2) in pH 7-9 buffers to form stable amide bonds. The side chain of lysine (K) residues and the N-terminus of each polypeptide contain a primary amine available for labeling. The sulfonated NHS ester of Sulfo-SHPP provides increased reagent solubility in comparison to the non-sulfonated form of Bolton-Hunter Reagent, which is no longer commercially available.

Procedure for Iodinating Purified Protein

The following protocol describes modification of a protein (avidin is the example) before iodination.[2]

Materials Required

- Modification Buffer: 200mM borate buffer, pH 9.0
- Avidin (Product No. 21121)
- Phosphate-buffered saline (PBS): 0.1M sodium phosphate, 150mM sodium chloride, pH 7.2 (Product No. 28372)

Method

1. Dissolve 190mg of avidin in 2mL of Modification Buffer.
2. Immediately before use dissolve 5mg of Water-Soluble Bolton-Hunter Reagent in 1mL of Modification Buffer. (Alternatively, add 0.5mg of the reagent directly to the avidin solution and then skip Step 3.)
3. Add 100µL of the Water-Soluble Bolton-Hunter Reagent solution to the avidin solution.
4. Incubate on ice for 3 hours with periodic mixing.
5. Remove the non-reacted Water-Soluble Bolton-Hunter Reagent by dialyzing against PBS or other suitable buffer.
6. Radiolabel with ^{125}I using Thermo Scientific™ Pierce™ Iodinating Reagents.

Protocol for Iodinating Cell Surface Proteins

The following general protocol uses Water-Soluble Bolton-Hunter Reagent (Thompson, *et al.*).

Materials

- DMSO
- Sodium phosphate buffer: 50mM sodium phosphate, pH 7.5
- Chloramine-T: just before use, prepare 5mg/mL chloramine-T in sodium phosphate buffer
- Hydroxyphenyl acetic acid: just before use, prepare 1mg/mL in water
- Sodium metabisulfite: just before use, prepare 12mg/mL in sodium phosphate buffer
- Borate buffer: 0.1M sodium borate, pH 8.3
- Cells: 10^7-10^9 cells/mL in Borate buffer.
- $Na^{125}I$
- Glycine buffer: 0.1M glycine in sodium borate buffer
- Phosphate-buffered saline (PBS): 100mM sodium phosphate, 150mM sodium chloride, pH 7.2 (Product No. 28372)

Method

1. Immediately before use, prepare 0.1mg/mL Water-soluble Bolton-Hunter reagent in DMSO. Place 1-20µL of this solution into a microcentrifuge tube.
2. The following reagents must be added in the specified order: quickly add 1-20µL of 100mCi/mL of $Na^{125}I$, 10µL of chloramine-T solution, 100µL of hydroxyphenyl acetic acid, and 10µL of sodium metabisulfite.
3. Add 100µCi of ^{125}I-labeled Bolton-Hunter Reagent to 1mL of cells and incubate for 30 minutes on ice.
4. Stop the reaction with 100µL of 0.1M glycine buffer.
5. Incubate cells for 15 minutes on ice.
6. Wash cells three times with PBS. This step removes any non-conjugated ^{125}I-labeled Bolton-Hunter Reagent and labeled internal components released by autolysis.

Related Thermo Scientific Products

28372	**BupH™ Phosphate Buffered Saline Packs,** 40 each
69576	**Slide-A-Lyzer™ MINI Dialysis Device Kit, 10K MWCO, 0.1mL**
43233	**Pierce Dextran Desalting Columns,** 5 × 10mL columns
28665	**Pierce Iodination Beads,** 50 beads
28601	**Pierce Iodination Tubes,** 10 tubes
28600	**Pierce Iodination Reagent,** 1g

General References

Bolton, A. E. and Hunter, W.M. (1973). The labelling of proteins to high specific radioactivities by conjugation to a ^{125}I-containing acylating agent. *Biochem J* **133:**529-39.

Feitelson, M.A., *et al.* (1981). Tryptic peptide mapping of picomolar quantities of protein labelled with the Bolton-Hunter Reagent. *Anal Biochem* **116:**473-9.

McCarthy, R.C., *et al.* (1988). Human prostatic acid phosphatase: purification, characterization, and optimization conditions for the radioimmunoassay. *Clin Chemica Acta* **132:**287-99.

Mock, D.M. and Dubois, D.B. (1986). A sequential, solid-phase assay for biotin in physiological fluids that correlates with expected biotin status. *Anal Biochem* **153:**272-8.

Raja, R.H., *et al.* (1984). Preparation of alkylamine and ^{125}I-radiolabelled derivatives of hyaluronic acid uniquely modified at the reducing end. *Anal Biochem* **139:**168-77.

Roll, F.J., *et al.* (1979). A new iodinating method of iodinating collagens for use in radioimmunoassay. *Anal Biochem* **96:**489-99.

Thirkell, D., *et al.* (1989). Serotype 8- and serocluster-specific surface-expressed antigens *of Ureaplasma urealyticum. Infect Immunity* **57:**1697-701.

Thompson, J.A., *et al.* (1987). Selective radiolabeling of cell surface proteins to a high specific activity. *Biochemistry* **26:**743-50.

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