INSTRUCTIONS

PDPH

22301

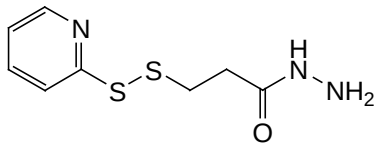
0505.3

Number	Description
22301	**PDPH** (3-[2-Pyridyldithio]propionyl hydrazide), 50mg

Formula: $C_{14}H_{15}N_3O_3$

Molecular Weight: 229.32

Spacer Arm: 9.2Å



Storage: Upon receipt store desiccated at 4°C. Product is shipped at ambient temperature.

Introduction

The Thermo Scientific PDPH is a heterobifunctional crosslinker containing sulfhydryl-reactive pyridyldithiol and carbonyl-reactive hydrazide moieties. Pyridyldithiols react with free sulfhydryls (-SH) to form disulfide bonds. Hydrazide groups react with carbonyls (aldehydes and ketones) to form stable hydrazone bonds. Aldehyde groups can be created by periodate-oxidation of sialic acid and other sugar components of glycoprotein polysaccharides. Thus, PDPH is useful for conjugating glycoproteins and sulfhydryl-containing peptides or proteins. Likewise, PDPH is useful as a sulfhydryl-addition reagent for glycoproteins and other carbohydrates; after reaction of the hydrazide to an oxidized carbohydrate, the pyridyldithiol group can be cleaved by a reducing agent to expose a sulfhydryl group. Yet another application for PDPH is to react the primary amine of the hydrazide moiety to a carboxyl group using the crosslinker EDC (Product No. 22980, see Related Thermo Scientific Products).

Important Product Information

- Hydrazides react with carbonyls most efficiently in amine-free, near-neutral conditions (pH 6.5-7.5). Carbonyls may exist at the reducing end of polysaccharides. To create additional carbonyls, oxidize sugar groups using either a specific oxidase, such as galactose oxidase, or 1-10mM sodium *meta*-periodate ($NaIO_4$; Product No. 20504). Oxidation with periodate is most efficient in acidic conditions (e.g., 0.1M sodium acetate, pH 5.5), although neutral buffers such as phosphate-buffered saline can be used. If oxidation is performed in acidic conditions, buffer exchange by dialysis or gel filtration (see Related Thermo Scientific Products) into neutral buffer may be necessary for optimal hydrazide reaction. Avoid Tris or other primary amine-containing buffers during glycoprotein oxidation and the hydrazide reaction as they react with the aldehyde groups, eliminating modification and conjugation of the intended biomolecules.
- The 2-pyridyldithio group of PDPH reacts optimally with free (reduced) sulfhydryls at pH 7-8. The reaction results in displacement of a pyridine-2-thione group, the concentration of which may be determined by measuring the absorbance at 343 nm (see Additional Information section). Reaction buffers must be free of thiols and disulfide reducing agents until quenching or reduction of the 2-pyridyldithiol is desired. To make target sulfhydryl groups available, reduce peptide disulfide bonds with Immobilized TCEP Disulfide Reducing Gel (Product No. 77712). Reduce protein disulfide bonds using 5-10mM DTT or TCEP solution (Product No. 77720), followed by desalting. Be aware that proteins (e.g., antibodies) may be inactivated by complete reduction of their disulfide bonds. Sulfhydryls can be added to primary amine sites using SATA (Product No. 26102) or 2-iminothiolane•HCl (Traut's Reagent, Product No. 26101).
- PDPH is most soluble in DMF and DMSO. Solubility limits in several solvents are listed in the Additional Information Section at the end of these instructions.

Example Protein Crosslinking Procedure

Assuming that buffer conditions are appropriate (see Important Product Information), conjugation reactions to both ends of this crosslinker can be performed simultaneously or sequentially (i.e., maleimide end followed by hydrazide end, or visa versa). The following procedure is an example of sequential conjugation between a sulfhydryl-containing protein (reacted to crosslinker first, then dialyzed) and a glycoprotein (oxidized with periodate, then dialyzed before addition to first protein).

A. Materials Required

- Coupling Buffer: 0.1M sodium phosphate, 0.15M NaCl, pH 7.2 (phosphate-buffered saline, PBS, Product No. 28372). This buffer is suitable for both pyridyldithiol and hydrazide coupling steps (see Important Product Information).
- Sulfhydryl Protein, reduced (see Important Product Information) and dissolved in Coupling Buffer
- Crosslinker (PDPH) vial, equilibrated to room temperature before opening
- Crosslinker Solvent: dimethylformamide (DMF, Product No. 20673) or dimethylsulfoxide (DMSO, Product No. 20688)
- Oxidation Buffer: 0.1M sodium acetate adjusted to pH 5.5
- Glycoprotein dissolved in Oxidation Buffer
- Sodium *meta*-periodate (Product No. 20504)
- Desalting columns or dialysis units for buffer exchange (see Related Thermo Scientific Products)

B. Sulfhydryl Protein Reaction with Crosslinker

Note: Perform Glycoprotein Oxidation (Section C) simultaneously.

1. Prepare 10-50mM Crosslinker in Solvent. Dissolving 2.3mg of PDPH in 1mL solvent yields a 10mM solution. Long-term stability of dissolved reagent is not known.
2. Add a volume of Crosslinker solution to the Sulfhydryl Protein to achieve a 5- to 10-fold molar excess of reagent over protein. To minimize protein damage or precipitation, do not exceed 10% DMF or DMSO in the final mixture.
3. Incubate reaction mixture for 2 hours at room temperature or 4 hours at 4°C.
4. Dialyze sample overnight against Coupling Buffer, or use a desalting column equilibrated with Coupling Buffer to remove excess reagent.

C. Glycoprotein Oxidation

1. Prepare 20mM periodate solution by dissolving 4.3mg of sodium *meta*-periodate per milliliter of Oxidation Buffer. Prepare a volume equal to the volume of Glycoprotein solution. Keep solution on ice and protect it from light.
2. Add 1mL of cold sodium *meta*-periodate solution to 1mL of the Glycoprotein solution and mix well. Allow the oxidation reaction to proceed in the dark for 30 minutes on ice or at 4°C. For more details, see instructions for Product No. 20504.
3. Dialyze oxidized glycoprotein solution overnight against Crosslinker Buffer, or use a desalting column equilibrated with Crosslinker Buffer to remove excess periodate and exchange the buffer.

D. Protein Conjugation

1. In proportions appropriate for the intended conjugation and number of available functional groups, combine solutions of crosslinker-modified Sulfhydryl Protein from Section B and the oxidized Glycoprotein from Section C.
2. Incubate reaction mixture for 2 hours at room temperature.
3. If desired, evaluate conjugation by SDS-PAGE analysis on a portion of the reaction mixture.
4. If desired, isolate conjugate from unconjugated proteins by size exclusion or ion exchange chromatography.

Additional Information

A. Solubility of PDPH

Table 1. Solubility limits of PDPH in various solvents

Solvent	Concentration mg/mL	Concentration mM
DMSO	140	610
DMF	177	773
Water	9.8	43
Ethanol	30.3	132
Methanol	58.9	257
Isopropyl alcohol	4.4	19
Dioxane	29.8	130
Acetonitrile	10.4	45
Methylene chloride	29.2	127
0.1M Sodium acetate, pH 5.5	14.2	62
Phosphate-buffered saline (PBS)	7.6	33

B. Protocol For Pyridine-2-Thione Assay to Determine Level of Sulfhydryl Modification

1. Dilute 100µL of PDPH-modified and desalted protein to 1mL with phosphate-buffered saline (PBS).
2. Measure and record the absorbance at 343nm of the protein sample compared to PBS blank (test in triplicate).
3. Add 10µL of 15mg/mL DTT (Product No. 20290) to the 1mL PDPH-modified protein sample; mix.
4. After exactly 15 minutes, measure and record the absorbance at 343nm of the reduced sample.
5. Calculate the change in absorbance: ΔA_{343} = (Ave. A_{343} after DTT) – (Ave. A_{343} before DTT)
6. Calculate the molar ratio of PDPH addition to protein using the following equation:

$$\frac{\Delta A}{8080} \times \frac{\text{MW of Protein}}{\text{mg/mL of Protein}} = \text{moles of PDPH addition per mole of Protein}$$

Where the value 8080 reflects the extinction coefficient for pyridine-2-thione at 343nm: 8.08 x 10^3 $M^{-1}cm^{-1}$.

Related Thermo Scientific Products

20036	**Bioconjugate Techniques,** 2nd edition, by Greg Hermanson, 2008, Academic Press, 1202 pages, softcover
66382	**Slide-A-Lyzer® Dialysis Cassette Kit,** 10K MWCO, 0.5-3mL capacity, 10 cassettes and accessories
89891	**Zeba™ Spin Desalting Columns, 7K MWCO, 5mL,** 5/pkg
22980	**EDC** [1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride], 5g

General References

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Greenfield, R.S., *et al.* (1990). Evaluation in vitro of adriamycin immunoconjugates synthesized using an acid-sensitive hydrazone linker. *Cancer Res* **50:**6600-7.

O'Shannessy, D.J., *et al.* (1984). A novel procedure for labeling immunoglobulins by conjugation to oligosaccharide moieties. *Immuno Lett* **8:**273-7.

Pain, D. and Surolia, A. (1981). Preparation of protein A-peroxidase monoconjugate using a heterobifunctional reagent, and its use in enzyme immunoassays. *J Immunol Meth* **40:**219-20.

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Ranadive, G.N., *et al.* (1993). A new method of Technetium-99 in labeling of monoclonal antibodies through sugar residues. A study with TAG-72 specific CC-49 antibody. *Nucl Med Biol* **20:**719-26.

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