INSTRUCTIONS

MPBH

22305

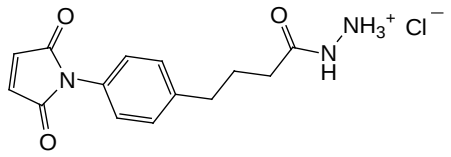
0523.4

Number	Description
22305	**MPBH** (4-[4-*N*-maleimidophenyl]butyric acid hydrazide hydrochloride), 50mg

Formula: $C_{14}H_{15}N_3O_3$•HCl + ½ dioxane

Molecular Weight: 353.80
(309.75 for HCl salt without dioxane)

Spacer Arm: 17.9Å



Note: The crystal structure of MPBH contains 1 mole of dioxane ($C_4H_8O_2$) per 2 moles of MPBH. Once the reagent is dissolved, the dioxane is no longer associated with the MPBH molecule.

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

Introduction

The Thermo Scientific MPBH is a heterobifunctional crosslinker containing sulfhydryl-reactive maleimide and carbonyl-reactive hydrazide moieties. Maleimides react with free sulfhydryls (-SH) to form stable thioether 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, MPBH is useful for conjugating glycoproteins and sulfhydryl-containing peptides or proteins. Alternatively, the hydrazide moiety can be reacted to carboxyl groups using the crosslinker EDC (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 to obtain efficient hydrazide reaction.
- Maleimides react with free (reduced) sulfhydryls at pH of 6.5-7.5 (at pH > 7.5, reaction to primary amines can occur). Reduce peptide disulfide bonds with Thermo Scientific 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 *N*-succinimidyl S-acetylthioacetate (SATA, Product No. 26102) or 2-iminothiolane•HCl (Traut's Reagent, Product No. 26101).
- MPBH is soluble in water, phosphate-buffered saline (PBS), DMF and DMSO. Solubility limits in several solvents are listed in the Additional Information Section at the end of these instructions.
- Avoid Tris or other primary amine-containing buffers during glycoprotein oxidation and the hydrazide reaction as they react with the aldehyde groups, preventing modification and conjugation of the intended biomolecules.
- EDC-mediated reactions are typically performed in MES buffer at pH 4.5-5 (Product No. 28390). Avoid buffers containing primary amines (e.g., Tris, glycine) or carboxyls (e.g., acetate, citrate), because they will quench the reaction. For more information about reaction conditions, see instructions for EDC, Product No. 22980.

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 maleimide and hydrazide coupling steps (See Important Product Information).
- Sulfhydryl Protein, reduced (See Important Product Information) and dissolved in Coupling Buffer
- Crosslinker (MPBH) 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 3.5mg of MPBH 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 (pH 7.2-8.5), or use a desalting column equilibrated with Coupling Buffer (pH 7.2-8.5) to remove excess reagent and exchange the buffer.

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 samples overnight against Coupling 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

Table 1. Solubility limits of MPBH in various solvents

Solvent	Concentration mg/mL	Concentration mM
DMSO	194	626
DMF	463	1490
Water	335	1080
Ethanol	18.7	60
Methanol	326	1050
Isopropyl alcohol	1.9	6.2
Dioxane	< 0.8	< 2.6
Acetonitrile*	< 0.7	< 2.2
Methylene chloride	< 1.4	< 4.5
0.1M Sodium acetate, pH 5.5	327	1050
Phosphate-buffered saline (PBS)	301	971

*MPBH is soluble in acetonitrile if a small amount of water is added.

Related Thermo Scientific Products

20036 **Bioconjugate Techniques,** 2nd edition, by Greg Hermanson, 2008, Academic Press, 1202 pages

66382 **Slide-A-Lyzer® Dialysis Cassette Kit, 10K MWCO, 3mL,** 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

Chamow, S.M., *et al.* (1992). Conjugation of soluble CD4 without loss of biological activity via a novel carbohydrate-directed crosslinking agent. *J Biol Chem* **267:**15916-22.

Ishi, Y. and Lehrer, S.S. (1986). Effects of the state of the succinimido-ring on the fluorescence and the structural properties of pyrene-maleimide-labeled a-tropomysin. *Biophys J* **50:**75-80.

Ishikawa, E., *et al.* (1983). Enzyme-labeling of antibodies and their fragments for enzyme immunoassay and immunohistochemical staining. *J Immunoassay* **4(3):**209-327.

Ishikawa, E., *et al.* (1983). Ultra sensitive enzyme immunoassay using fluorogenic, luminogenic, radioactive and related substances and factors to limit the sensitivity. *Proceedings 2nd Internat Sym Immunoenzymatic Tech*.

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

O'Shannessy, D.J. and Wilcheck, M. (1990). Immobilization of glycoconjugates by their oligosaccharides: Use of hydrazide-derivatized matrices. *Anal Biochem* **191:**1-8.

Current product instructions are available at *www.thermoscientific.com/pierce*. For a faxed copy, call 800-874-3723 or contact your local distributor.