INSTRUCTIONS

# BMPH, EMCH and KMUH

## 22297 22106 22111

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| Number | Description |
|---|---|
| **22297** | **BMPH** (*N*-[ß-maleimidopropionic acid] hydrazide, trifluoroacetic acid salt), 50mg<br>Formula: $C_7H_9N_3O_3 \cdot CF_3CO_2H$<br>Molecular Weight: 297.19<br>Spacer Arm: 8.1Å |
| **22106** | **EMCH** (*N*-[ε-Maleimidocaproic acid) hydrazide, trifluoroacetic acid salt), 50mg<br>Formula: $C_{10}H_{15}N_3O_3 \cdot CF_3CO_2H$<br>Molecular Weight: 339.27<br>Spacer Arm: 11.8Å |
| **22111** | **KMUH** (*N*-[κ-maleimidoundecanoic acid] hydrazide, trifluoroacetic acid salt), 50mg<br>Formula: $C_{15}H_{25}N_3O_3 \cdot CF_3CO_2H$<br>Molecular Weight: 409.40<br>Spacer Arm: 19.0Å |

**Storage:** Upon receipt store desiccated at 4°C. Reagents are shipped at ambient temperature.

## Introduction

Thermo Scientific BMPH, EMCH and KMUH are heterobifunctional crosslinkers 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, these crosslinkers are useful for conjugating glycoproteins and sulfhydryl-containing peptides or proteins. Alternatively, the amine of the hydrazide moiety can be reacted to carboxyl groups 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 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).
- 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.

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## 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 (BMPH, EMCH or KMUH) 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, 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. For KMUH, 4.1mg/mL equals 10mM solution. For EMCH, 3.4mg/mL equals 10mM solution. For BMPH, 3.0mg/mL equals 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% Crosslinker Solvent 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 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 for 30 minutes in the dark on ice or at 4°C. For more details, see instructions for Product No. 20504.
3. Dialyze samples 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.

## Related Thermo Scientific Products

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| **20002** | **Bioconjugate Techniques,** by Greg Hermanson, 1996, Academic Press, 785 pages, softcover |
| **66382** | **Slide-A-Lyzer™ Dialysis Cassette Kit, 10K MWCO, 3mL** |
| **89891** | **Zeba™ Spin Desalting Columns, 5mL,** 5/pkg. |
| **22980** | **EDC** [1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride], 5g |

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