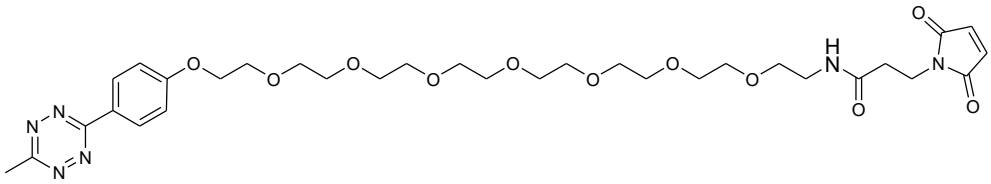
Methyltetrazine-PEG7-maleimide Kit (BP-43846)

Product Name: Methyltetrazine-PEG7-maleimide kit

Catalog number: BP-43846

Chemical Structure:



Formular: $C_{32}H_{46}N_6O_{11}$

Molecular Weight: 690.74

Solubility: DMSO, DMF, DCM

Appearance: Vial 1 Violet-Red solid; Vial 2 White solid; Vial 3 Liquid

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

Shelf life: for each component, at least 12 months at -20°C

Important Note: *Methyltetrazine-PEG7-maleimide degrades quickly (hours) at room temperature. This product is provided as a two-component kit. The stock solution of Methyltetrazine-PEG7-maleimide is prepared in situ.*

Introduction

Methyltetrazine-PEG7-maleimide is mainly used to crosslink two biomolecules together. The maleimide reacts with thiol-containing compound at pH 6.5 to 7.0 to activate the molecules, then the methyltetrazine will react with trans-cyclooctene (TCO)-containing compound to yield the conjugates.

Note:

• pH for the thiol-maleimide reaction: 6.5-7.5.

The maleimide group reacts predominantly with free sulfhydryl's to form thioether. At pH > 7.0, primary amines will also react with maleimide, and hydrolysis of the maleimide groups will occur. At pH 7, the maleimide group is ~1,000 times more reactive toward a free sulfhydryl than

to an amine.

• Thiol and tetrazine free: no thiol and tetrazine residual in biomolecules and buffers.

Additional Materials Required

• Solvent: dimethyl sulfoxide (DMSO) or dimethyl formamide (DMF)

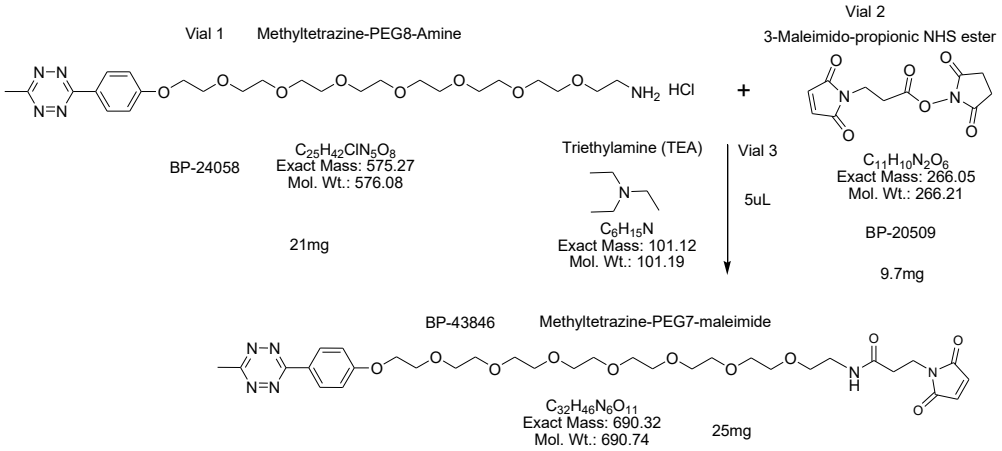
• Reducing reagents: TCEPT or Immobilized TCEP disulfide reducing gel

• Reaction buffer: PBS buffer (pH 6.5-7.0) with 5-10 mM EDTA

• (Optional) quenching buffer: concentrated (0.5-1 M) cysteine, DDT or other thiol containing reducing agents

• Spin Desalting Columns

Preparation of Methyltetrazine-PEG7-maleimide Stock Solution



1. Add 3ml of dry DMF or DMSO to Methyltetrazine-PEG8-Amine (vial #1) and shake till the compound dissolved.

2. Add the solution above slowly to 3-Maleimido-propionic NHS ester (vial #2, white solid) slowly, added 5uL of TEA (Vial #3) stir for about 2 hrs at room temperature. The progress of the reaction can be followed by TLC and/or utilization of liquid Chromatography-Mass Spectrometry (LCMS).

3. Stock solution of Methyltetrazine-PEG7-maleimide is ready to use. At this stage the product is stable if stored at -20C or lower for short periods of time (hours).

4. The concentration of Methyltetrazine-PEG7-maleimide stock solution is about 12mM, i.e. is

amount: 36umol.

Monitoring Methodology:

Thin Layer Chromatography (TLC): methylene chloride 1:20 or 4 ml: 10 drops, silica gel normal phase plate developed with a potassium permanganate spray. e.g. the Retention factor (Rf) of the Methyltetrazine-PEG7-Maleimide is slightly lower than the maleimide-NHS esters. When the reaction is complete, it will be one clean spot on the plate.

Liquid Chromatography Mass Spectrometry (LCMS): 3-Maleimido-propionic NHS ester and/or Methyltetrazine-PEG8-Amine in the spectra will indicate completion of the reaction and formation of Methyltetrazine-PEG7-maleimide.

Procedure for Labeling Proteins

1. If required, buffer exchange the IgG1 antibody sample into PBS at ~2 mg/mL by using a spin desalting column.

2. Add TCEP stock solution to the IgG1 antibody solution at final concentration of 2.5mM, pipette up and down several times to mix.

3. Incubate the reaction for 1-2 hours at 37ºC.

4. Buffer exchange into reaction buffer to remove excess TCEP.

Note: EDTA need be added to reaction buffer to a final 5-10 mM to avoid S-S bind reformation.

5. Add a 9x freshly prepared maleimide reagent above to the protein sample.

6. Incubate reaction mixture for 1-4 hour at room temperature or for 2-8 hours at 4°C.

Note: many IgG1 antibody will precipitate or structural variation when the DMF or DMSO concentration exceeds 20% of the final reaction volume.

7. Remove the excess reagent by desalting the labeled protein through a spin desalting column or by dialysis.

Procedure for click reaction

Please check a corresponding protocol in the PEG linkers for click chemistry for details.