USER GUIDE

Azido-PEG3-Maleimide Kit

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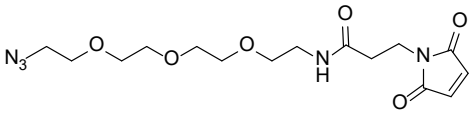
Cat. No.	CCT-AZ107
Chemical Structure	
Chemical Composition	$C_{15}H_{23}N_5O_6$
Molecular Weight	369.37
Solubility	DMSO, DMF, DCM
Appearance	Vial 1: Off-white to grey solid Vial 2: Slightly yellow oil
Storage	Upon receipt store at -20°C. Product shipped at ambient temperature
Shelf life	At least 12 months at -20°C

Copper-free Click Reaction

1. Add dry water-miscible organic solvent such as dimethyl sulfoxide (DMSO) or dimethyl formamide (DMF) to Azido-PEG_3-amine (vial #2) and shake for about 30 seconds.

Kit size	Solvent amount
25 mg	1 mL
100 mg	2.5 mL
1000 mg	25 mL

2. While keeping the Maleimide-NHS ester (vial #1, white solid) under a dry atmosphere (e.g. with nitrogen) slowly add a solution of Azido-PEG_3-Amine (vial #2) with stirring or shaking, and then stir or shake for 30 minutes at room temperature. The progress of the reaction can be followed by TLC.
3. Stock solution of Azido-PEG_3-Maleimide is ready to use. At this stage the product is stable if stored at -20°C or lower for short periods of time (hours).
4. The concentration of azide-PEG4-maleimide stock solution is:

Kit size	Conc. of Azido-PEG3-Maleimide	Amount of Azido-PEG3-Maleimide
25 mg	75 mM	0.075 mmol
100 mg	120 mM	0.3 mmol
1000 mg	120 mM	3 mmol

5. TLC data: The solvent is typically something like methanol: methylene chloride 1:20 or 4 mL: 10 drops, run on a silica gel normal phase plate and developed with a potassium permanganate spray. E.g. the R_f of the Azido-PEG_3-Maleimide is slightly lower than the one of the Maleimide-NHS ester. When the reaction is complete, it will be one clean spot on the plate.

Procedure for Labeling Proteins

1. If required, buffer exchange the protein sample into phosphate reaction buffer at 1-5 mg/mL using a spin desalting column.
2. Add stock solution of TCEP to the protein solution at final concentration of 20 mM, pipette up and down several times to mix.
3. Incubate the reaction to for 30 minutes.
4. Buffer exchange TCEP reduced protein into reaction buffer. If a reaction buffer does not contain EDTA, add immediately stock solution of EDTA to a solution of reduced protein at final concentration of 5-10 mM.
5. Add a 20-fold molar excess of *freshly prepared* maleimide reagent to the protein sample.
6. Incubate reaction mixture for 1-4 hours at room temperature or for 2-8 hours at 4°C.

   Note: Many proteins will precipitate when the DMF or DMSO concentration exceeds 10% of the final reaction volume; if protein solubility is not an issue, there is no limit to the DMF or DMSO concentration that may be used.
7. Remove the excess reagent by desalting the labeled protein through a spin desalting column or by dialysis.

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