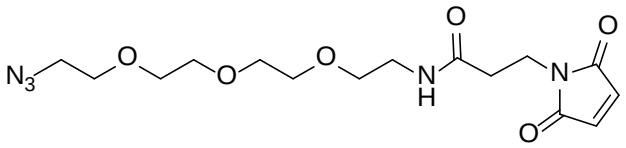
Click Chemistry Tools
Bioconjugate Technology Company

http://www.clickchemistrytools.com
tel: 480 584 3340
fax: 866 717 2037

Azido-PEG$_3$-Maleimide Kit

Product No.:	AZ107
Product Name:	Azido-PEG$_3$-Maleimide
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 month at -20^{0}C

Important Product Information

- Molecules to be reacted with Azido-PEG$_3$-Maleimide must have free (reduced) sulfhydryls.
- Do not use buffers that contain sulfhydryl-containing components (e.g., DTT), and azides
- The maleimide group reacts predominantly with free sulfhydryls at pH 6.5-7, forming stable thioether bonds. At pH values > 7.0, reactivity toward primary amines and hydrolysis of the maleimide groups can occur. At pH 7, the maleimide group is ~1,000 times more reactive toward a free sulfhydryl than to an amine.

Additional Materials Required

- Water-miscible organic solvent such as dimethyl sulfoxide (DMSO) or dimethyl formamide (DMF)
- Reducing reagents such as TCEPT or Immobilized TCEP Disulfide Reducing Gel
- Reaction buffer: Phosphate-buffered saline (PBS) or other sulfhydryl-free buffer at pH 6.5-7.0. Include 5-10 mM EDTA to help prevent the re-oxidation of disulfides by trace divalent metals.
- (Optional): Quenching buffer: concentrated (0.5-1 M) cycteine, DDT or other thiol containing reducing agents
- Spin Desalting Columns

Preparation of Azido-PEG$_3$-Maleimide Stock Solution

1. Add dry water-miscible organic solvent to Azido-PEG$_3$-amine (vial #2) and shake for ca. 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 TLC.
3. Stock solution of Azido-PEG$_3$-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 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 one of the Maleimide-NHS ester. And 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 hour 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.

Procedure for click reaction

In order to perform the Cu(I)-catalyzed azide-alkyne click reaction (CuAAC) at least one additional component is required – copper(I) catalyst. However, the general thermodynamic instability of Cu(I), which results in its easy oxidation to inactive Cu (II) usually requires the copper catalyst to be prepared with an appropriate chelating ligand.

The selection of partial protocol for CuAAC reaction depends mainly on two factors, including nature of alkyne-modified biopolymer (e.g. cell lysis, fixed cells), availability of chelating ligand.

Click Chemistry Tools offers reaction kits that enable researchers to perform Cu(I)-catalyzed azide-alkyne click reaction. The kits contain detailed procedure and all reagents required to perform up to 25 CuAAC reactions.

For research use only

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