DATA SHEET

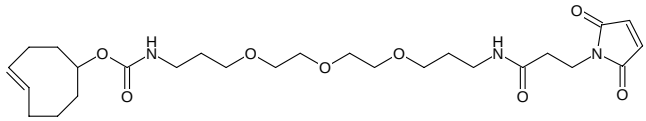
interchim

Jena Bioscience

TCO-PEG$_3$-Maleimide

trans-Cyclooctene-PEG$_3$-Maleimide

Cat. No.	Amount
CLK-1002-10	10 mg
CLK-1002-100	100 mg



Structural formula of TCO-PEG$_3$-Maleimide

For research use only!

Shipping: shipped at ambient temperature

Storage Conditions: store at -20 °C

Shelf Life: 12 months after date of delivery

Molecular Formula: $C_{26}H_{41}N_3O_8$

Molecular Weight: 523.62 g/mol

CAS#: 1609659-01-9

Purity: ≥ 90 % (HPLC)

Form: colorless to slightly yellow oil

Solubility: Chloroform, DCM, DMF, DMSO

Applications:
Protein-protein conjugates

Protein-antibody conjugates

Peptide-small molecule conjugates

^{18}F radiolabelling

Protein-oligonucleotide conjugates

Surface modification

Description:
The inverse-electron demand Diels-Alder cycloaddition reaction of *trans*-Cyclooctenes (TCO) with tetrazines is a bioorthogonal reaction that possesses exceptional kinetics (k > 800 $M^{-1}s^{-1}$) and selectivity. Such excellent reaction rate constants are unparalleled by any other bioorthogonal reaction pair described to date.
The extremely fast kinetics of this reaction enables rapid conjugation (30 min or less) two low abundance biopolymers (e.g. 5 µM) in aqueous buffered media. The TCO-tetrazine click reaction is the standard in protein-protein conjugation.

Advanced Features

- By far, the fastest kinetics among any other bioorthogonal reaction pairs
- Reactions complete in 30-60 minutes at low protein concentrations (5-10 µM)
- TCO functional group remains stable in aqueous buffered media (weeks at 4°C, pH 7.5)
- Conjugation efficiency > 99 % without requiring a toxic catalyst (e.g. Cu(I))

Important Product Information

- Molecules to be reacted with maleimide compounds must have free (reduced) sulfhydryls.
- Do not use buffers that contain sulfhydryl-containing components (e.g. DTT) or azides.
- The maleimide group reacts predominantly with free sulfhydryls at pH 6.5-7.5, forming stable thioether bonds. At pH values > 7.5, 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 Material Required

- Water-miscible organic solvent such as dimethyl sulfoxide (DMSO) or dimethyl formamide (DMF)
- Reaction buffer: Phosphate-buffer (100 mM sodium phosphate, 150 mM NaCl, pH 7.5) or other suitable amine-free buffer at pH

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6.5-7.5. Include 5-10 mM EDTA to help prevent the reoxidation of disulfides by trace divalent metals.
- TCEP
- Spin Desalting Colum (e.g. ThermoScientific Zeba™)

Procedure for Labeling Proteins
- Buffer exchange proteins into phosphate reaction buffer at 1 - 5 mg/ml using a desalt spin column.
- Immediately before use prepare 5-20 mM TCO-PEG$_3$-Maleimide reagent in DMSO or DMF.
- Add a 20-fold molar excess maleimide reagent to the protein sample and incubate for 1-4 hours at room temperature or for 2-8 hours at 4°C.
- **Note:** The reaction solution may appear cloudy as a result of the low aqueous solubility of TCO-PEG$_3$-Maleimide; usually, such solutions become clearer as the reaction proceeds. 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.
- Remove excess reagent by desalting the labeled protein through a desalt spin column or by dialysis.

Long-term Aqueous Stability of TCO-labeled Samples

TCO-PEG$_3$-modified goat IgG (17.2 DOL) losses about 10.5 % of its reactivity toward tetrazines when stored at 4°C for 4 weeks and 7 % when stored at -20°C in 100 mM sodium phosphate, 150 mM sodium chloride solution at pH 7.5. For long-term storage azide and thiol-containing buffers should be avoided.

Protein-Protein Tetrazine/TCO Conjugation
- Calculate volume tetrazine-labeled protein (1 - 5 mg/ml) equivalent to a 1.1 - 5 fold molar excess over desired volume TCO-labeled protein (1 - 5 mg/ml).
- Mix calculated volume tetrazine-labeled protein with desired volume of TCO-labeled protein.
- Allow reaction to proceed for 60 minutes at room temperature.
- Store conjugate at 4°C until ready for purification or use.

Troubleshooting

Problem: No or poor labeling of protein with TCO
- Possible reason: One or more sample is not labeled
  - Confirm molecules were labeled or repeat activation process
- Possible reason: TCO-PEG$_3$-Maleimide decomposed
  - Allow product to equilibrate to room temperature before opening
  - Prepare new solutions in the indicated dry solvents
  - Avoid buffers that contain sulfhydryl
- Possible reason: Excess reagent not quenched or removed
  - Remove non-reacted reagent by dialysis or desalting

Problem: Low conjugation efficiency
- Possible reason: Suboptimal reaction conditions
  - Increase incubation time
  - Optimize conjugation conditions by altering molar excess
  - Perform conjugation reactions at 37°C

Selected References:

Devaraj *et al.* (2009) Fast and Sensitive Pre-Targeted Labeling of Cancer Cells through a Tetrazine/trans-Cyclooctene Cycloaddition. *Angew. Chem. Int. Ed.* **48**:7013.

Haun *et al.* (2009) Probing Intracellular Biomarkers and Mediators of Cell Activation Using Nanosensor and Bioorthogonal Chemistry. *ACS Nano.* **5**:3204.

Blackman *et al.* (2008) Tetrazine Ligation: Fast Bioconjugation Based on Inverse-Electron-Demand Diels-Alder Reactivity. *J. Am. Chem. Soc.* **130**:13518.

Devaraj *et al.* (2008) Tetrazine-Based Cycloadditions: Application to Pretargeted Live Cell Imaging. *Bioconjugate Chem.* **19**:2297.