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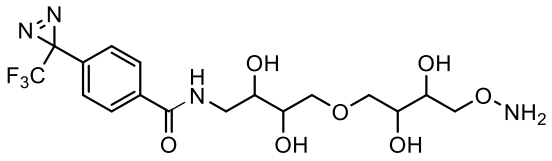
Phenyldiazirine oxLink™ (T2A10)

Lyophilized powder, 250 µg, ≥95% pure by HPLC

Product Number: CM81402-250UG and CM81402-4x250UG
CAS Registration Number: N/A



Chemical Formula: $C_{17}H_{23}F_3N_4O_7$
Exact Mass: 452.15
Molecular Weight: 452.39

Product Description

CellMosaic's Phenyldiazirine oxLink™ (T2A10) is a proprietary photo-crosslinking reagent developed at CellMosaic for studying biomolecular interactions. This reagent combines the highly efficient carbene-generating phenyldiazirine group with convenient oxime reversible chemical crosslinking and the super hydrophilic AqT™ linker. These structural features make it highly advantageous to use this reagent over traditional photo-crosslinking reagents.

1) Photo-crosslinking group: oxLink™ uses a trifluoromethyl phenyldiazirine group, a highly efficient carbene-generating photo-crosslinking group. Trifluoromethyl phenyldiazirine photolyzes around 360 nm, at which photodamage to biomolecules is minimized. The generated carbene inserts C–H bonds into the neighboring biomolecular partner within picoseconds. Because the electron-withdrawing trifluoromethyl group confers stability on the intermediate diazo-isomer, no side products are detected under normal labeling conditions.
2) Reversable chemical crosslinking group: oxLink™ reagent contains an aminooxy group for chemical crosslinking of the biomolecule containing a ketone or aldehyde group. Aminooxy-ketone ligation is orthogonal to peptide chemistry and will allow highly selective solid phase-based separation of the aminooxy-labeled crosslinked products. In particular, the selective purification method will permit the characterization of protein complexes in complex matrices, such as plasma, cellular membranes, and cell lysates, where these samples may contain free thiols that interfere with the labeling and purification processes. **Figure 1** shows a workflow of how a reversible oxLink™ can be used to crosslink the interacting biomolecule partner and identify the crosslinking site or detect the interaction partner.
3) Hydrophilic AqT™ linkers: AqT™ linkers are novel proprietary biomaterials invented at CellMosaic that are chemically assembled from a class of natural and edible sugar alcohol (SA) compounds with properties by design. As the trifluoromethyl phenyldiazirine group is highly hydrophobic, biomolecules labeled with trifluoromethyl phenyldiazirine using a traditional ethylene and ethylene glycol-type linker tend to aggregate and destabilize the labeled protein. T2A10 AqT™ linker is made by linking two threitols together, greatly increasing the hydrophilicity of oxLink™ (**Figure 2**) and its water solubility (>27 mg/mL). The molecule is also more biocompatible with decreased non-specific hydrophobic interactions with other biomolecules, allowing high loading of phenyldiazirine groups.

Application of the Product

- Labeling biomolecules containing aldehyde or ketone groups
- Studying dynamic biomolecule interactions via photo-crosslinking (e.g., studying biological complexes and networks, protein-protein interactions, protein-DNA interactions, small ligand-protein interactions)

Key Features of the Product

- Highly water soluble (>27 mg/mL) and biocompatible
- High loading with minimized aggregation
- Contains orthogonal peptide chemical crosslinking group capable of forming a reversible linkage
- Contains an efficient carbene-generating photo-crosslinking group

Storage/Stability

- Stable at RT in the dark. Recommended storage of the product is below -20°C, which is viable for several years without any sign of decomposition

Usage

Lyophilized solid and ready to use after dissolving in water or labeling buffer (use slightly acidic buffer for labeling)

References:

Protein crosslinking reviews: a) Brunner, J. (1993) *Annu. Rev. Biochem*. **62**, 485−514. b) Freedman, R. B. (1979) *Trends Biochem. Sci*. 193–197. c) Herrmann, J. M., Westermann, B., Neupert, W. (2001) *Methods Cell Biol.* **65**, 217–230. d) Fancy, D. A. (2000) *Curr. Opin. Chem. Biol.* **4**, 28–32. e) Fasold, H., Klappenberger, J., Meyer, C., Remold, H. (1971) *Angew. Chem. Internat. Edit*. **10**, 795–801. f) Bayley, H. Photogenerated Reagents in Biochemistry and Molecular Biology, Vol. 12. Elsevier, Amsterdam, Neth, 1983.

3-Trifluoromethyl-3-phenyldiazirine reference: Brunner, J., Senn, H. & Richards, F. M. (1980) J. Bio. Chem. **255**, 3313−3318.

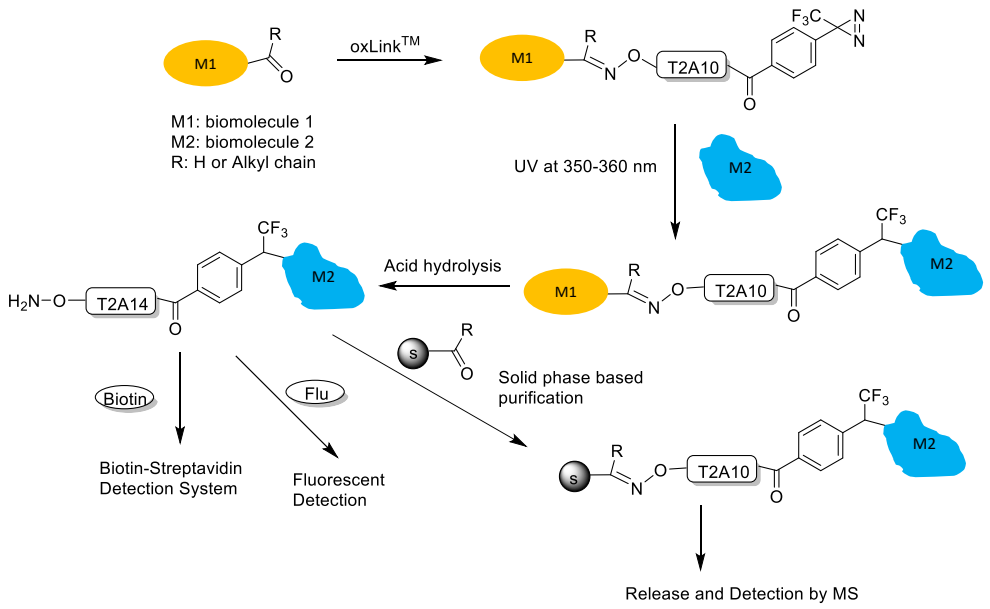


Figure 1. oxLink™ workflow using water-soluble Phenyldiazirine oxLink™ (T2A10). oxLink™ is tethered to a target biomolecule of interest (M1) with an artificially introduced ketone or aldehyde group via the aminooxy functional group. The resulting oxime bond can be hydrolyzed, releasing the photo-crosslinked biomolecule (M2) with a free aminooxy group. M2 can then react with any aldehyde- or ketone-containing molecule, such as solid phase, biotin, or fluorescent dye, for further purification, detection, and identification.

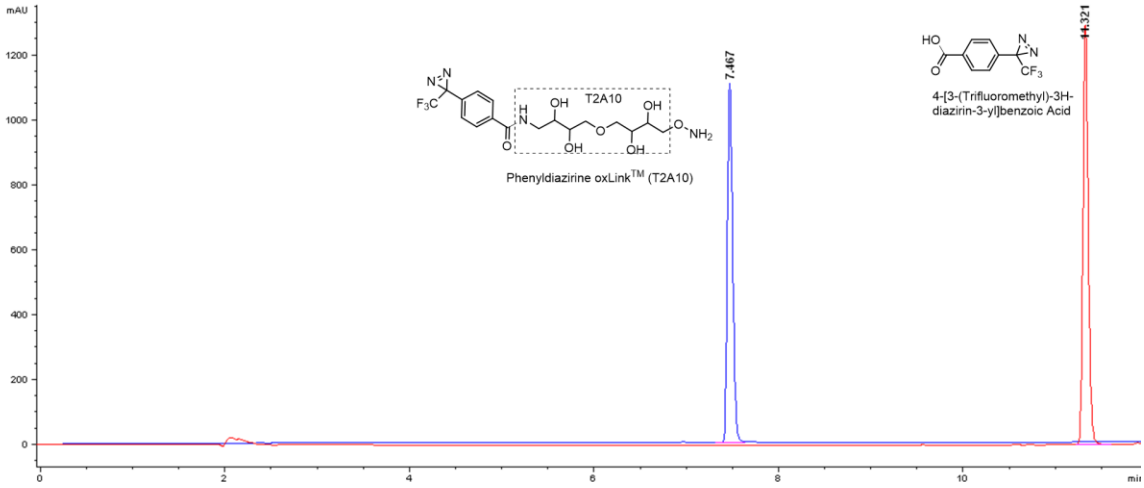


Figure 2. C18 HPLC analysis of 4-[3-(trifluoromethyl)-3H-diazirin-3-yl]benzoic acid (TFDB, red, in DMSO) and oxLink™ (blue, in water). HPLC method utilizes 0.1% TFA in water as buffer A and 0.1% TFA in acetonitrile as buffer B, with 5% to 95% B within 12 minutes. The unmodified TFDB requires close to 75% acetonitrile to elute it out from the column and is insoluble in water despite containing a hydrophilic carboxylic acid end group. With only two SA units attached to the phenyldiazirine, oxLink™ (T2A10) is easily soluble in water (> 27 mg/mL, no high limit has been tested) and requires 29% less acetonitrile to elute it out from the column.

Important Notes & Contact Information

READ BEFORE USING ANY RESOURCES PROVIDED HEREIN

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Sample data are provided for illustrative and example purposes only and represent a small dataset used to verify kit performance in the CellMosaic laboratory. Information about the chemicals and reagents used in the kit are provided as necessary.

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