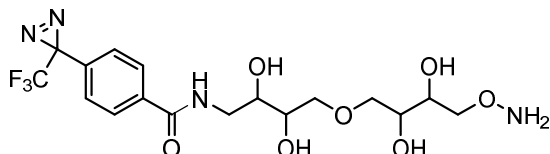


Phenyldiazirine oxLink™ (T2A10)

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

Product Number: CM81402-250UG and CM81402-4x250UG

CAS Registration Number: N/A



Chemical Formula: C₁₇H₂₃F₃N₄O₇
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) Reversible chemical crosslinking group: oxLink™ reagent contains an aminoxy group for chemical crosslinking of the biomolecule containing a ketone or aldehyde group. Aminoxy-ketone ligation is orthogonal to peptide chemistry and will allow highly selective solid phase-based separation of the aminoxy-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.

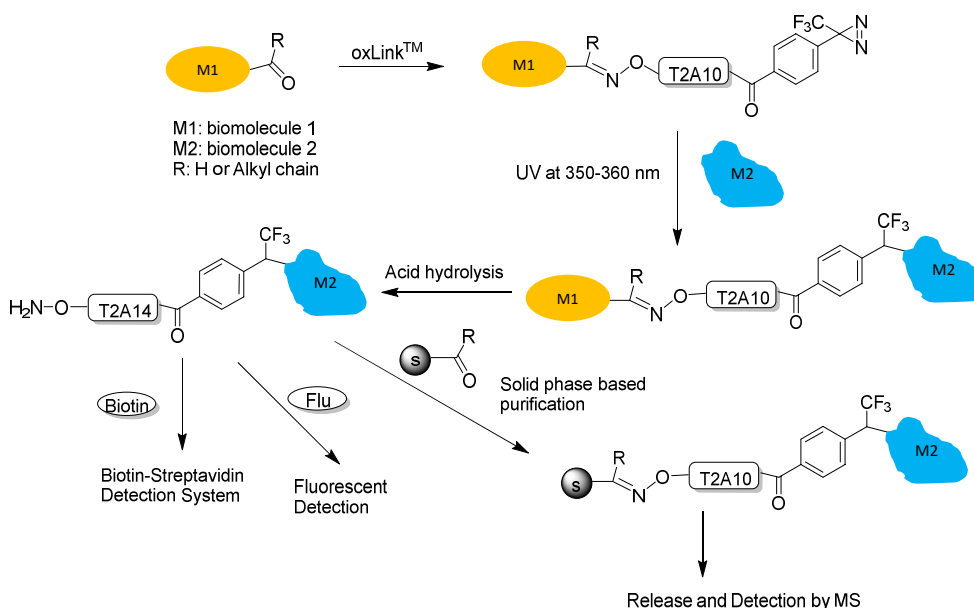


Figure 1. oxLink™ workflow using water-soluble Phenyldiazirine oxLink™ (T2A10). oxLink™ is tethered to a target biomolecule of interest (M1) with an artificially introduced ketone or aldehyde group via the aminoxy functional group. The resulting oxime bond can be hydrolyzed, releasing the photo-crosslinked biomolecule (M2) with a free aminoxy 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.

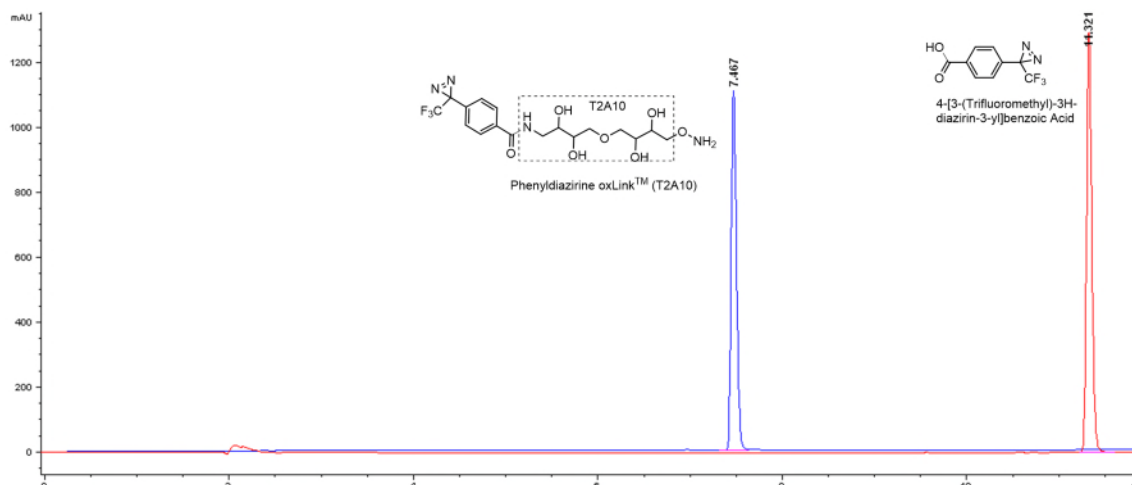


Figure 2. C18 HPLC analysis of 4-[3-(trifluoromethyl)-3H-diazirin-3-yl]benzoic acid (TFDB, red, in DMSO) and oxLink™ (blue, in water). HPLC method utilizes 0.1% TFA in water as buffer A and 0.1% TFA in acetonitrile as buffer B, with 5% to 95% B within 12 minutes. The unmodified TFDB requires close to 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

The information provided in this document and the methods included in this package are for information purposes only. CellMosaic provides no warranty of performance or suitability for the purpose described herein.

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.

For Research Use Only. Not for Use in Diagnostic Procedures.

The information in this document is subject to change without notice. CellMosaic assumes no responsibility for any errors that may appear in this document. In no event shall CellMosaic be liable, whether in contract, tort, warranty, or under any statute or on any other basis for special, incidental, indirect, punitive, multiple, or consequential damages in connection with or arising from this document, including but not limited to the use thereof.

NOTICE TO PURCHASER: LIMITED LICENSE

The purchase of this product includes a limited, non-transferable license to use this product to practice the labeling methods using the reagents solely for the purchaser's research activities. The license granted herein is personal to the original purchaser and may not be transferred to any other party outside the purchaser's company. No other right or license is conveyed or granted either expressly, by implication or by estoppel, to resell or repackage this product. Further information can be obtained by contacting:

Director of Licensing
c/o CellMosaic, Inc.
10-A Roessler Road, Woburn, MA 01801.
Phone: 781-463-0002
Fax: 781-998-4694
E-mail: info@cellmosaic.com