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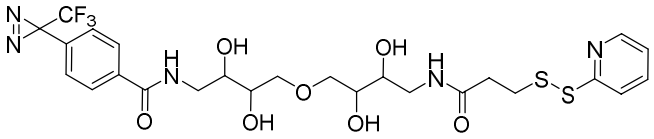
Phenyldiazirine sxLink™ (T2A14)

Lyophilized powder, 250 µg, ≥95% pure by HPLC
One vial of dissolution buffer is included

Product Number: CM81401-250UG and CM81401-4x250UG
CAS Registration Number: N/A



Chemical Formula: $C_{25}H_{30}F_3N_5O_7S_2$
Exact Mass: 633.15
Molecular Weight: 633.66

Product Description

CellMosaic's Phenyldiazirine sxLink™ (T2A14) is a proprietary photo-crosslinking reagent developed at CellMosaic for studying biomolecular interactions. This reagent combines the highly efficient carbene-generating phenyldiazirine group with a cleavable disulfide crosslinking group via a super hydrophilic AqueaTether™ (AqT™) based linker. These structural features make it highly advantageous to use this reagent over traditional photo-crosslinking reagents.

1) Photo-crosslinking group: sxLink™ 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: sxLink™ reagent contains a reversible disulfide group for chemical crosslinking of the biomolecule containing a free thiol. In combination with site-directed cysteine mutagenesis, a thiol-cleavable photo-crosslinker has been extensively used in Khorana's laboratory to study rhodopsin and transducing interactions. **Figure 1** shows a workflow for how a reversible sxLink™ 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 a trifluoromethyl phenyldiazirine using a traditional ethylene and ethylene glycol-type linker tend to aggregate and destabilize the labeled protein. T2A14 AqT™ linker is made by linking two threitols together, greatly increasing the hydrophilicity of sxLink™ (**Figure 2**) and its water solubility (2.2 mg/mL saturated solution). 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 free thiol 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

- More hydrophilic than traditional photo-crosslinking reagent, soluble, and biocompatible
- High loading with minimized aggregation
- 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

Procedure

1. Take one tube out and warm to ambient temperature.
2. Add 100 µL or more of dissolution buffer and vortex for 30 seconds to dissolve.
3. Centrifuge briefly to ensure no liquid is in the cap. Stock solution of sxLink™ (2.5 mg/mL or lower) is prepared and ready to use.
4. Dilute the stock solution in your labeling 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.

Thiol cleavable photo-crosslinking reagents for studying rhodopsin and transducing interactions: a) Resek, J. F., Bhattacharya, S. & Khorana, H. G. (1993) J. Org. Chem. 58, 7598−7601. b) Resek, J. F., Farrens, D. & Khorana, H. G. (1994) Proc. Natl. Acad. Sci. USA 91, 7643−7647. c) Cai, K, Itoh, Y. & Khorana, H. G. (2001) Proc. Natl. Acad. Sci. USA 98, 4877−4882. d) Huang Y, Khorana HG. (2003) Mapping of Contact Sites in Interaction between Transducin and Light-Activated Rhodopsin. Presented at 17th Symposium of the Protein Society, July 26–30, Boston, Massachusetts.

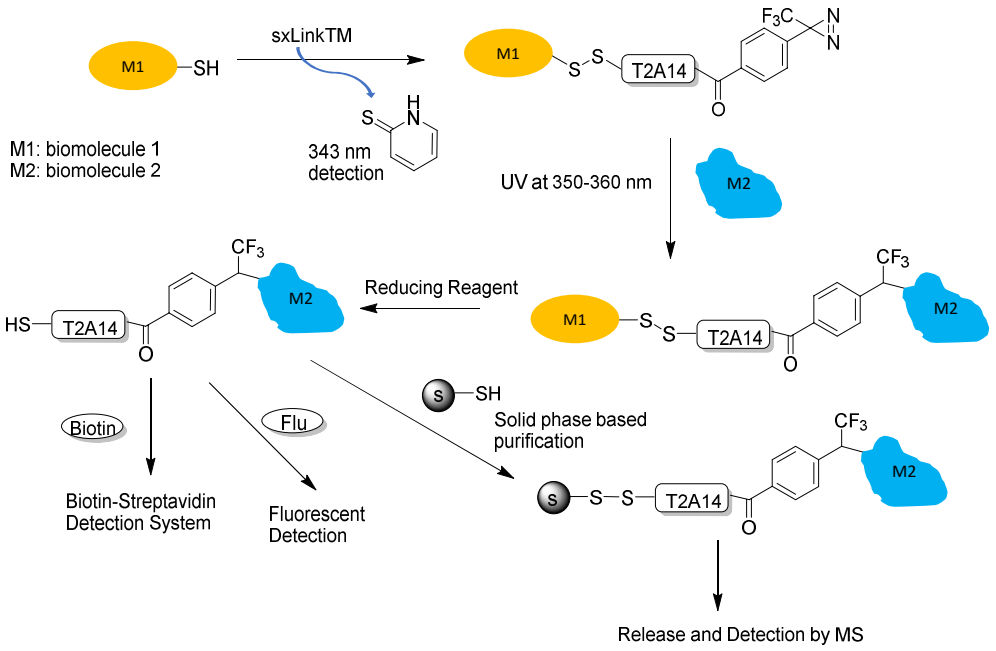


Figure 1. sxLink™ workflow using water-soluble Phenyldiazirine sxLink™ (T2A14). sxLink™ is tethered to a target biomolecule of interest (M1) via the chemical disulfide crosslinker (thiol exchange). The progression of labeling can be followed by UV analysis of the released chromophore 2-thiolpyridone at 343 nm. The modified biomolecule (M1) is then reconstituted into its native complex with biomolecule M2. A phenyldiazirine group is activated by UV irradiation, resulting in a crosslink with the neighboring biomolecule (M2). Finally, the disulfide bond connecting the two biomolecules is cleaved by a reducing reagent, revealing the biomolecule interaction by transfer of the free thiol to M2.

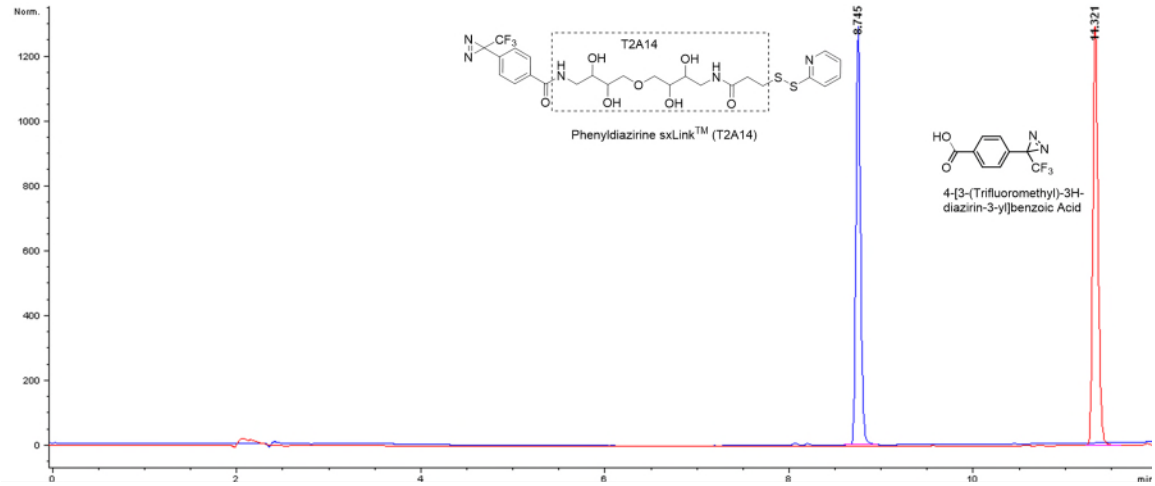


Figure 2. C18 HPLC analysis of 4-[3-(trifluoromethyl)-3H-diazirin-3-yl]benzoic acid (TFDB, red, in DMSO) and sxLink™ (blue, in water). HPLC method utilizes 0.1% TFA in water as buffer A and 0.1% TFA in acetonitrile as buffer B, with 5% to 95% B within 12 minutes. The unmodified TFDB requires close to 75% acetonitrile to elute it out from the column and is insoluble in water despite containing a hydrophilic carboxylic acid end group. Phenyldiazirine sxLink™ (T2A14) has the other end capped with the very hydrophobic pyridyl disulfide. Despite being linked with two extremely hydrophobic groups, sxLink™ (T2A14) still elutes earlier than TFDB (19% less acetonitrile) and has a water solubility of 2.2 mg/mL (saturated solution).

Important Notes & Contact Information

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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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