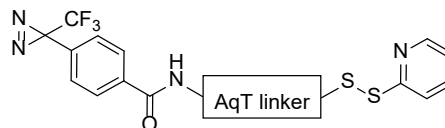


Phenyldiazirine sxLink™

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

MW: 633.66

Product Description

CellMosaic's Phenyldiazirine sxLink™ is a proprietary photo-crosslinking reagent developed by CellMosaic for studying biomolecular interactions. This reagent combines a highly efficient, carbene-generating phenyldiazirine group with convenient oxime-based reversible chemical crosslinking and the super-hydrophilic AqT® linker. These structural features make this reagent highly advantageous compared to 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: The sxLink™ reagent contains a reversible disulfide group for chemical crosslinking with biomolecules containing free thiols. In combination with site-directed cysteine mutagenesis, thiol-cleavable photo-crosslinkers have been extensively used in Khorana's lab to study rhodopsin and transducing interactions. **Figure 1** illustrates a workflow showing how a reversible sxLink™ can be used to crosslink 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. Because the trifluoromethyl phenyldiazirine group is highly hydrophobic, biomolecules labeled with it using a traditional ethylene and ethylene glycol-type linkers tend to aggregate and destabilize the labeled protein. The AqT® linker greatly enhances the hydrophilicity and water solubility of sxLink™ (2.2 mg/mL). It also improves biocompatibility and reduces 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
- Enable high loading with minimized aggregation
- Reversible linkage
- Includes an efficient carbene-generating photo-crosslinking group

Storage/Stability

- Stable at room temperature when kept in the dark.
- Recommended storage below -20°C, where the product remains stable for several years without signs of decomposition

Procedure

1. Remove one tube and allow it to warm to ambient temperature.
2. Add 100 µL (or more) of dissolution buffer and vortex for 30 seconds to dissolve.
3. Briefly centrifuge to ensure no liquid remains in the cap. A stock solution of sxLink™ (2.5 mg/mL or lower) is now prepared and ready for use.
4. Dilute the stock solution with your labeling buffer as needed 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.

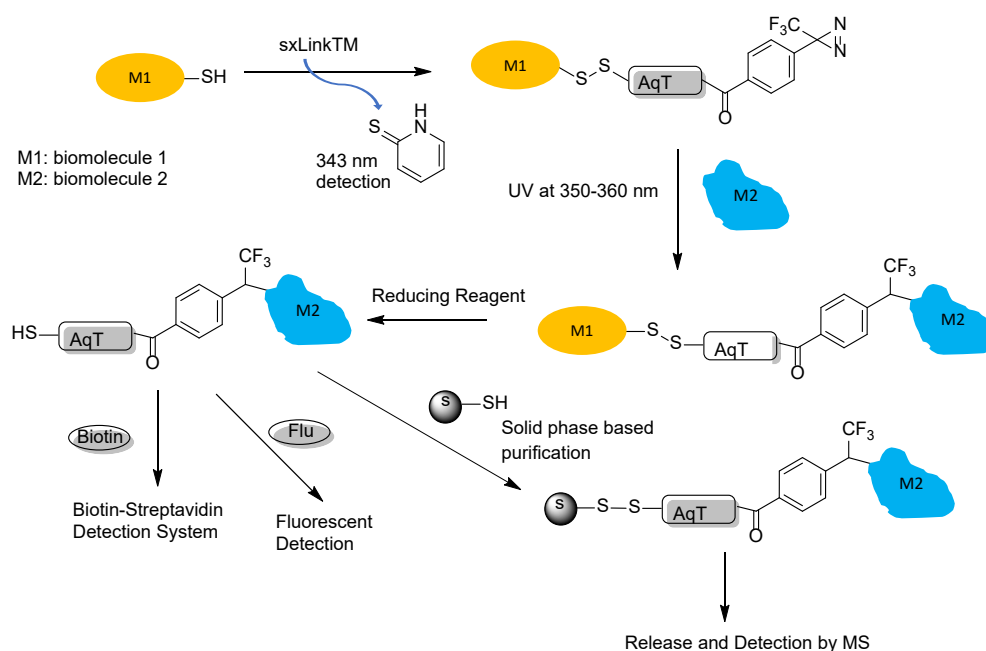


Figure 1. sxLink™ workflow using water-soluble Phenyldiazirine sxLink™. sxLink™ is tethered to a target biomolecule of interest (M1) via a chemical disulfide crosslinker through thiol exchange. The progression of labeling can be monitored by UV analysis of the released chromophore, 2-thiopyridone, at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

Important Notes & Contact Information

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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