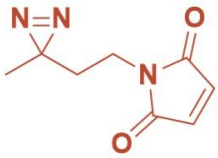
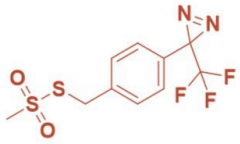
General Protocol

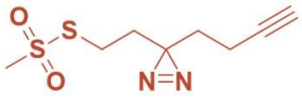
PRODUCTS	TAG-TRANSFER PRODUCTS CYSTEINE SPECIFIC DIAZIRINE PHOTOCROSSLINKERS
SAMPLE SIZE	**25 mg**
CATALOGUE NUMBERS	**RBM-0000768 - Maleimido Alkyl Diazirine** **RBM-0000767 (MTS reagent) - Tag-Transfer TFM Diazirine** **RBM-0000766 (MTS reagent) - Tag-Transfer Alkynyl Diazirine** **RBM-0000765 (MTS reagent) - Tag-Transfer Alkyl Diazirine**



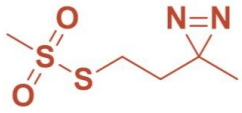
Maleimido Alkyl Diazirine



Tag-Transfer
TFM Diazirine



Tag-Transfer
Alkynyl Diazirine



Tag-Transfer Alkyl
Diazirine

Notes to user

- Compounds and all subsequently labelled proteins should be handled in the dark (i.e. by exclusion of the light) as much as possible and stored at -20°C in amber vials or vials wrapped in aluminium foil.
- The products are soluble in DMSO or DMF to at least 10 mg/ml.
- Labelling of cysteine residues should be performed in buffer containing a small amount <10% of DMSO or DMF.
- With the MTS labels (RBM-0000765-767) the methanethiosulfonate creates a disulphide bond upon reaction with cysteine, so the system should not contain free

Suggested Protocol

1. Prepare 5 mM solution of the crosslinker in DMSO. For example, this corresponds to dissolving 1 mg of crosslinker RBM-0000768 in 1.12 ml of DMSO. This stock solution can be aliquoted and frozen. It is stable for at least several weeks.
2. To a solution of 100 µl of 100 µM protein in buffer (pH 6-9) add 10 µl of 5 mM crosslinker solution (5x molar excess) and allow to react for 5-10 minutes.
3. Desalt to remove excess crosslinker.
4. Add the cross-linker labelled protein to its interacting partner or a mixture containing the potential interacting partner and irradiate with 365 nm UV light. Depending on the intensity of the light source, the irradiation can take 1-30 min.
5. Analyze the products using SDS-PAGE or HPLC and identify/isolate the cross-linked products.
6.For the MTS reagents, the isolated crosslinked product can be dissociated using DTT or 2-mercaptoethanol which reduces the disulphide bond. The cross-linker will detach from the protein that was originally labelled, but will still remain attached to its interacting partner. Since the crosslinker now contains a free thiol group, this can be further explored for labelling with suitably functionalized fluorophore to visualize it, biotin for easy isolation or iodoacetamide for location of exact crosslinked site by mass spectrometry.