UbiQ
targeting the ubiquitin system

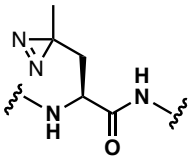


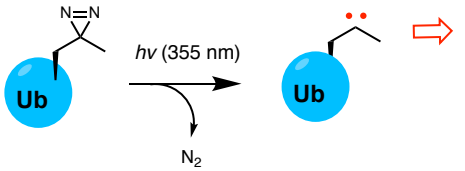
Figure 1. photoLeu

Ub photoLeu8 *(human sequence, recombinant)*

UbiQ code	:	UbiQ-153
Batch #	:	B01062019-001
Amount	:	100 ug, lyophilized powder
Purity	:	≥95% by RP-HPLC
Mol. Weight	:	8.58 kDa
Storage	:	upon arrival, powder at −20°C; solution at −80°C. Please avoid multiple freeze/thaw cycles and store dark.

Productsheet

Background. UbiQ-153 is a crosslinking reagent based on ubiquitin in which Leu8 is replaced by the photoreactive analog photoleucine (photoLeu, ^{p}L, Figure 1).[1] Upon irradiation of the diazirine with UV light, a very reactive carbene species is formed (Figure 2). Unspecific binding is minimal for carbene based crosslinking[1-4] because carbenes are quenched quickly by water, meaning only residues that are very nearby (and thereby contribute to tight binding) will react covalently. Thus any unbound UbiQ-153 will react with water before being able to undergo non-specific reactions with other proteins.



Ub photoLeu

Figure 2.

MQIFVKT^{p}LTGKTITLE
VEPSDTIENVKAKIQD
KEGIPPDQQRLIFAGK
QLEDGRTLSDYNIQKE
STLHLVLRLRGG

important: sample preparation

- dissolve the powder in as little DMSO as possible (e.g. 20 - 40 mg/mL)
- add the DMSO stock slowly to milliQ (please note the order of addition)
- buffer the aq. solution as desired
- a final buffered stock of 0.5 mg/mL (59 uM) will contain 1.25 vol% DMSO when prepared from a 40 mg/mL DMSO stock.
- under normal laboratory light, UbiQ-153 is photo-stable

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K48- and K63-linked polyUb pLeu73) chain assembly (see reference 1 for details).

- enzymatically synthesized K48-, and K63-linked Ub chains were assembled by combining a E1-E2-E3 blocked Ub mutant with UbiQ-153.
- Ub can be blocked for the E1-E2-E3 pathway by several modifications: introducing a tag on the C-terminus or by removing Gly76.
- K48-linked Ub chains were obtained from a reaction containing 10 ug of blocked Ub and 100 ug of UbiQ-153 (Ub^{pL}), 8 nM E1 (UBA1), 4 uM E2-25K, 4 mM TCEP, and 15 mM ATP in 100 uL 50 mM Tris pH 8.0 buffer incubated at 37°C for 20 hours.
- in a similar fashion, reactions to generate K63-linked Ub chains contained 30 uM of each Ubc13 and Uev1a with same monomers in addition to 50 ng of UbK63R to influence chain length.
- Following the completion of each reaction, Ub chains with a His tag were diluted into a volume of 40 mL HisTrap buffer A (20 mM phosphate, 200 mM NaCl, 10 mM imidazole, pH 7.4), and loaded onto a 5 mL HisTrap column.
- side products of the reaction flowed through the columns and $polyUb^{pL}$ chains with a His6 tag in the proximal position were eluted in HisTrap buffer B (20 mM phosphate, 200 mM NaCl, 280 mM imidazole, pH 7.4).
- $polyUb^{pL}$ reactions without Ub-His6 were first passed through a 1 mL GST column in PBS pH 7.4 buffer to remove E1 and E2 enzymes.
- defined polymers of $polyUb^{pL}$ can be resolved on a Superdex 75 16/60 size exclusion column (GE Life Sciences) in PBS, pH 7.4.
- fractions containing the desired chain lengths were confirmed with SDS-PAGE and stored at -20°C until needed.

crosslinking conditions

- crosslinking was performed in 96 well-plates, allowing for a 30 minute pre-incubation at 30°C.
- samples were placed 5 - 10 cm from the light source and UV-irradiated for 30 min
- for optimal crosslinking please test various distances from the UV lamp

Literature. (1) Chojnacki et al. *Cell Chem Biol* **2017**, *24*, 443. (2) Liang et al. *Angew Chem Int Ed* **2017**, *56*, 2744. (3) Zhou et al. *Nat Comm* **2016**, *7*, article 10589. (4) Dubinsky et al. *Bioorg Med Chem* **2012**, *20*, 554.