UbiQ
targeting the ubiquitin system

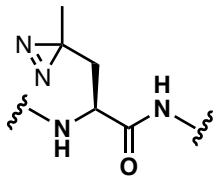


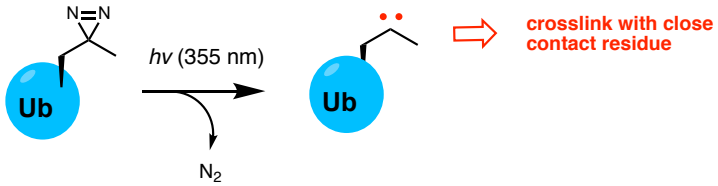
Figure 1. photoLeu

Ub photoLeu73 *(human sequence, recombinant)*

UbiQ code	:	UbiQ-154
Batch #	:	B01032017-001
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.

Productsheet

Background. UbiQ-154 is a new crosslinking reagent based on ubiquitin in which Leu73 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-154 will react with water before being able to undergo non-specific reactions with other proteins.



Ub photoLeu

Figure 2.

Sequence

MQIFVKTLTGKTITLEVEPSDTIENVKAKIQDKEGIPPDQQRLIFAGKQLEDGRTLSDYNIQKESTLHLVLR^{P}LRGG

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**
- **for example, 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.**
- **if desired, the DMSO can be removed from the buffered stock by dialysis or 3 kDa spin-filters**

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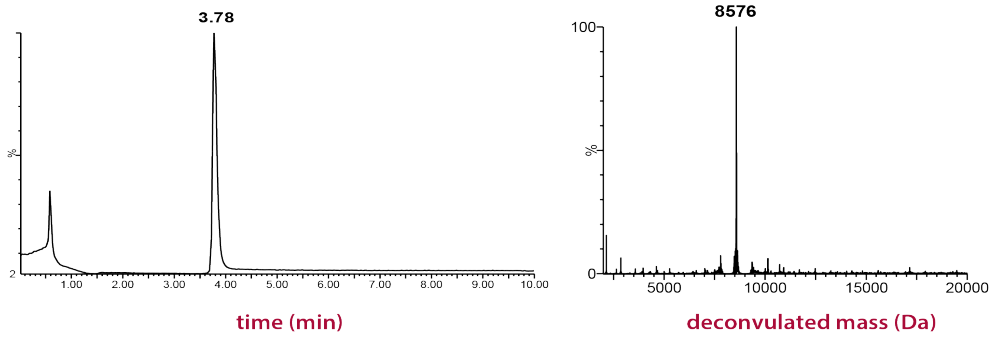

LC-MS analysis. Mobile phase A = 1% CH_3CN, 0.1% formic acid in water (milliQ) and B = 1% water (milliQ) and 0.1% formic acid in CH_3CN. XBridge BEH300 C18 5µm 4.6x100mm; flow rate = 0.8 mL/min, runtime = 10 min, column T = 40°C. Gradient: 30% ⇨ 60% B over 6.5 min.

Example of 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-154
- 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-154 (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.
- under normal laboratory light, UbiQ-154 is photo-stable

Crosslinking Conditions

- crosslinking was performed in 96 well-plates, allowing for a 30 minute preincubation at 30°C.
- samples were placed 10 cm from the light source and UV-irradiated for 30 min using 5X8W UV Bulbs 302/355 nm (Cleaver Scientific – UV Crosslinker).

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.