UbiQ
targeting the ubiquitin system

**His6-3C-MAP1LC3a-Dha** *(human sequence, semi-synthetic)*

| | | |
|---|---|---|
| UbiQ code | : | UbiQ-158 |
| Batch # | : | B01092016-001 |
| Amount | : | 50 ug, lyophilized powder |
| Purity | : | ≥95% by RP-HPLC |
| Mol. Weight | : | 16.18 kDa |
| Storage | : | upon arrival powder at −20°C; solution at −80°C. Please avoid multiple freeze/thaw cycles. |

# Productsheet

**Background.** UbiQ-158 is an activity-based probe for E1 activating enzymes and E2 conjugating enzymes that are involved in autophagy (e.g. ATG7 and ATG3, respectively). UbiQ-158 is based on the MAP1LC3a protein sequence in which the C-terminal Gly has been replaced by a dehydroalanine residue (Dha). The N-terminus is labeled with an His6 affinity tag and a 3C protease cleavage site (QG), Cys17 has been mutated to a Ser residue (S). The design of UbiQ-158 allows it to be processed in a native manner by E1-E2-E3 enzymes that recognize MAP1LC3a and during this process it forms an electrophilic intermediate that can react with an active-site Cys residue in the E1-E2-E3 cascade, thereby creating a covalent bond.

**A**

HHHHHH-SAALEVLFQGPG-MPSDRPFKQR RSFADRSKEV QQIRDQHPSK IPVIIERYKG EKQLPVLDKT KFLVPDHVNM
SELVKIIRRR LQLNPTQAFF LLVNQHSMVS VSTPIADIYE QEKDEDGFLY MVYASQETF-**Dha**

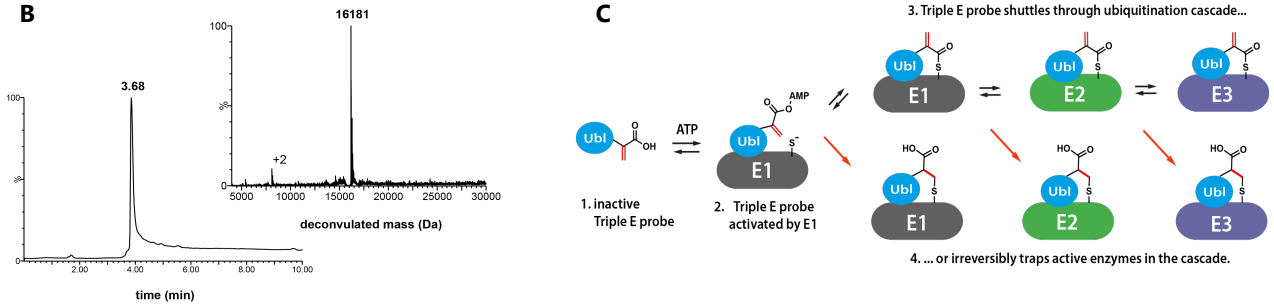


**Figure 1 –** A: sequence. B: LC-MS analysis. Mobile phase A = 1% $CH_3CN$, 0.1% formic acid in water (milliQ) and B = 1% water (milliQ) and 0.1% formic acid in $CH_3CN$. XBridge BEH300 C18 5µm 4.6x100mm; flow rate = 0.8 mL/min, runtime = 10 min, column T = 40°C. Gradient: 30-60%B over 6.5 min. C: mode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

## Important: general sample preparation

- prepare a 50 mg/mL stock of probe in 100% DMSO (=1.00 mg probe in 20 µL DMSO)
- dispense the DMSO stock slowly into 0.56 mL $ddH_2O$
- when homogenised, add 100 mM NaCl: *e.g. by adding 12 uL of a 5M NaCl stock*
- next, buffer to 50 mM NaOAc pH 4.5: *e.g. add 8.5 uL of 3.5M stock*
- the NaOAc buffered stock (total volume by now= 0.6 mL, 1.67 mg/mL= 103 uM) can be subsequently buffered to >pH 7: *e.g. add 100 mM HEPES pH 7.5 from a 1M stock (60 uL)*
- total volume by now= 0.66 mL, 1.52 mg/mL= 94 uM)
- all aliquots can be snap-frozen and stored at −80°C

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## General Experimental Conditions

E1 labeling assay

- E1 (1 µM) in 50 mM HEPES pH 8, 100 mM NaCl, 1mM DTT, 10 mM $MgCl_2$ and 250 uM ATP was incubated with probe (30 µM, 0.5 mg/mL) at 37°C for >30 min.
- for a negative control, omit ATP
- the reaction was quenched by the addition of reducing sample buffer and heating (90°C for 10 min).

E1+E2 labeling assay

- E1 (0.63 µM) and E2 enzyme (2.5 µM) in 50 mM HEPES pH 7.5, 100 mM NaCl, 1mM DTT, 5 mM $MgCl_2$ and 250 uM ATP were incubated with probe (12.5 µM, 0.2 mg/mL) at 37°C for >30 min.
- for a negative control, omit ATP
- the reaction was quenched by the addition of reducing sample buffer and heating (90°C for 10 min).

E1+E2+(HECT/RBR) E3 labeling assay

- E1 (0.63 µM), E2 enzyme (2.5 µM) and E3 enzyme (0.5 µM) were incubated with probe (50 µM, 0.8 mg/mL) in 50 mM HEPES pH 7.5, 100 mM NaCl, 1mM DTT, 5 mM $MgCl_2$ and 250 uM ATP at 30°C for 2h.
- for a negative control, omit ATP
- the reaction was quenched by the addition of reducing sample buffer and heating (90°C for 10 min).

Notes

1. to find optimal reaction conditions one can test an ATP concentration of 250 uM, 1 mM and 5 mM
2. in general labeling is better when TCEP (100 - 250 uM) is used as reducing agent (please do not add too much TCEP as the Dha warhead can react with phosphines like TCEP)

Cell lysate labeling*

- example of lysis buffer: 50 mM HEPES pH 7.5, 5 mM $MgCl_2$, 100 mM NaCl, 0.5 mg/mL CHAPS, 100 uM TCEP and protease inhibitor cocktail (Roche, EDTA free).
- 100 µg of fresh lysate is incubated with probe (>0.5 ug) and 5 mM ATP at 37°C for >1h.
- fresh 1 mM ATP and 1 mM $MgCl_2$ are added every 20 minutes
- for a negative control, lysates are treated with 2 units of Apyrase (Sigma Aldrich) prior to addition of the probe (ATP and $MgCl_2$ are omitted).
- the reaction was terminated by the addition of 3x Loading Buffer (Invitrogen) containing beta-mercaptoethanol.

* besides this protocol, in which probe is added to freshly prepared lysate, performing the labeling during the lysis procedure can result in better labeling as it allows trapping enzymes that rapidly lose activity during lysis.

**Literature.** (1) Mulder et al. *Nat Chem Biol* **2016**, *12*, 523.

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