UbiQ
targeting the ubiquitin system

His6-3C-MAP1LC3a-Dha *(human sequence, recombinant protein precursor from E. coli)*

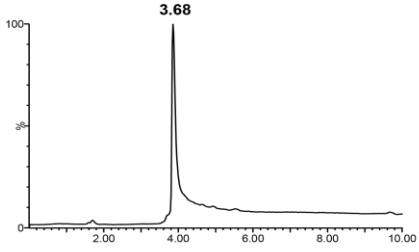
UbiQ code	:	UbiQ-158
Batch #	:	B01092016-001
Amount	:	50 ug, lyophilized powder
Purity	:	≥95% by RP-HPLC
Mol. Weight	:	16.18 kDa
Storage	:	upon arrival powder at –20°C; solution at –80°C. Please avoid multiple freeze/thaw cycles.

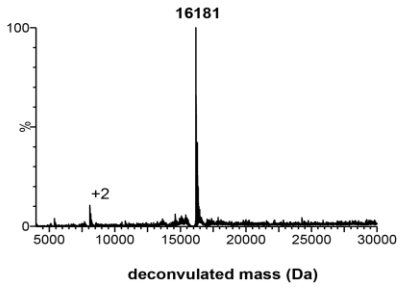
Productsheet

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 UbiQ-158 is based on the MAP1LC3a protein sequence in which the C-terminal Gly has been replaced by a dehydroalanine residue (**Dha**).[1] The *N*-terminus is labeled with an **His6** affinity tag and a 3C protease cleavage site (**QG**), Cys17 has been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.

Sequence

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





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.

Important: sample preparation

- **dissolve the powder in as little DMSO as possible (e.g. 40 mg/mL)**
- **add this DMSO stock slowly to milliQ (please note the order of addition)**
- **buffer the aq. solution as desired (final stocks of e.g. 0.5 mg/mL will contain 1.25 vol% DMSO)**
- **buffer exchange using 3 kDa spin filters or dialysis membrane allows total removal of DMSO if desired.**
- **since the probe functions as a native MAP1LC3a protein, E1-E2 (and E3) reaction conditions can be based on those of native MAP1LC3a.**
- **in order to optimize the labeling conditions, one can vary the ATP concentration (250 uM - 5 mM) and perform labeling with or without a reducing agent, such as DTT.**

Literature. (1) (a) Mulder et al. *Nat. Chem. Biol.* **2016**, *12*, 523. (b) MPC Mulder, F. El Oualid and H. Ovaa. Adenylation enzyme inhibitors. Application WO/2016/032332 and NL2015/050596

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