UbiQ
targeting the ubiquitin system

Ub-Dha *(human sequence, synthetic)*

UbiQ code	:	UbiQ-101
Batch #	:	B01042015-001
Amount	:	50 ug, lyophilized powder
Purity	:	≥95%
Mol. Weight	:	8.58 kDa
Storage	:	upon arrival, powder at –20°C; solution at –80°C. Please avoid multiple freeze/thaw cycles.

Productsheet

Background. UbiQ-101 (Ub-Dha) is an activity-based probe for Ub E1, E2 and (HECT/RBR) E3 ligases. It is based on ubiquitin (Ub) in which the C-terminal Gly76 has been replaced by a dehydroalanine (Dha) residue. It is processed in a native manner by Ub E1, E2 and (HECT/RBR) E3 ligases and during this process it forms an electrophilic intermediate that can react with the active site Cys residue of the E1, E2 and (HECT/RBR) E3 enzyme, thereby creating a covalent bond (Figure 1C).

important: sample preparation

- dissolve the powder in as little DMSO as possible (20 - 40 mg/mL)
- add the DMSO stock to milliQ (please note the order of addition) and mix
- buffer the aq. solution as desired
- For full details please see open-access reference 1: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5108872/

General Experimental Conditions E1 labeling assay

UBE1 or UBA6 (1 µM) in 50 mM HEPES pH 8, 100 mM NaCl, 10 mM $MgCl_2$ and 250 uM ATP was incubated with probe (30 µM) at 37°C for 30 min. The reaction was quenched by the addition of reducing sample buffer and heating (if required). Samples can be analyzed by SDS-PAGE using Coomassie staining.

General Experimental Conditions E2 labeling assay

E2 enzyme (2.5 µM) and UBE1 (0.63 µM) in 50 mM HEPES pH 7.5, 100 mM NaCl, 5 mM $MgCl_2$ and 250 uM ATP were incubated with probe (12.5 µM) at 37°C for 30 min. The reaction was quenched by the addition of reducing sample buffer and heating (90°C for 10 min). Samples can be analyzed by SDS-PAGE using Coomassie staining.

General Experimental Conditions HECT E3 labeling assay

Nedd4L (2.5 µM), UBE2D (0.5 µM) and UBE1 (0.25 µM) were incubated with probe (50 µM) in 50 mM HEPES pH 7.5, 100 mM NaCl, 5 mM $MgCl_2$ and 250 uM ATP at 30°C for 2h. The reaction was quenched by the addition of reducing sample buffer and heating (90°C for 10 min). Samples can be analyzed by SDS-PAGE using Coomassie staining.

Please note optimal reaction conditions can vary between E2 and E3 enzymes.

It is advised to vary the ATP concentration from 250 uM to 5 mM and determine which is best for your experiment.

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

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Rabobank
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BIC/SWIFT: RABONL2U

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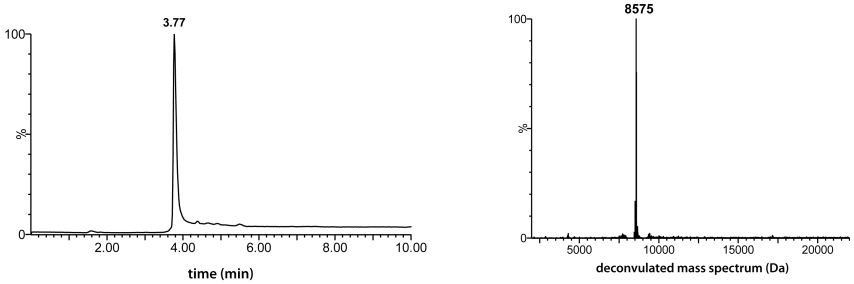
A

MQIFVKTLTGKTITLEVEPSDTIENVKAKIQDKEGIPPDQQRLIFAGKQLEDGRTLSDYNIQKESTLHLVLRLRG-Dha

B

C



D

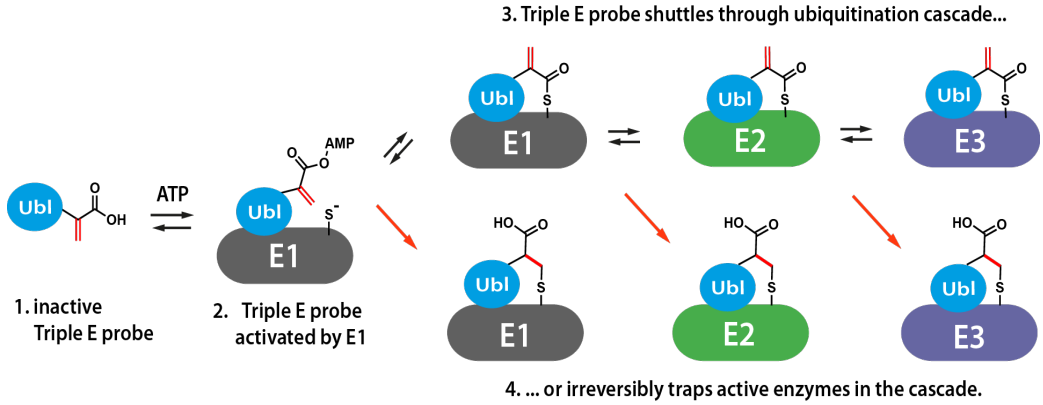


Figure 1. A: sequence. B: SDS-PAGE analysis. 12% Bolt Bis-Tris Plus gel (Life technologies) and MES running buffer. CBB staining with Coommassie G-250. C: LC-MS analysis. Mobile phase A= 1% CH_3CN, 0.1% formic acid in milliQ and B= 1% milliQ and 0.1% formic acid in CH_3CN. XBridge BEH300 C18 5µm 4.6x100mm; column T= 40°C, flow= 0.8 mL/min. Gradient: 30–60%B over 6.5 min. D: Mode of action Ub-Dha activity-based probes.

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