UbiQ
targeting the ubiquitin system

His6-Ahx-Ahx-Ub-Dha *(human sequence, synthetic)*

UbiQ code	:	UbiQ-103
Batch #	:	B01042016-001
Amount	:	50 ug, lyophilized powder
Purity	:	≥95%
Mol. Weight	:	9.64 kDa
Storage	:	upon arrival, powder at –20°C; solution at –80°C. Please avoid multiple freeze/thaw cycles.

Productsheet

Background. UbiQ-103 is an activity-based probe for Ub E1, E2 and (HECT/RBR) E3 ligases. It is based on ubiquitin (Ub) in which Gly76 has been replaced by a dehydroalanine (Dha) residue. The N-terminus is labeled with a His6 tag and two 6-aminohexanoic acid (Ahx) linkers are used to create extra space between the His6 and Ub protein for efficient access of His6 binding entities. UbiQ-103 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 1).

A

HHHHHH-Ahx-Ahx-
MQIFVKTLTGKTITLEVEPSDTIENVKAKIQDKEGIPPDQQRLIFAGKQLEDGRTLSDYNIQKESTLHLVLRLRG-Dha

B

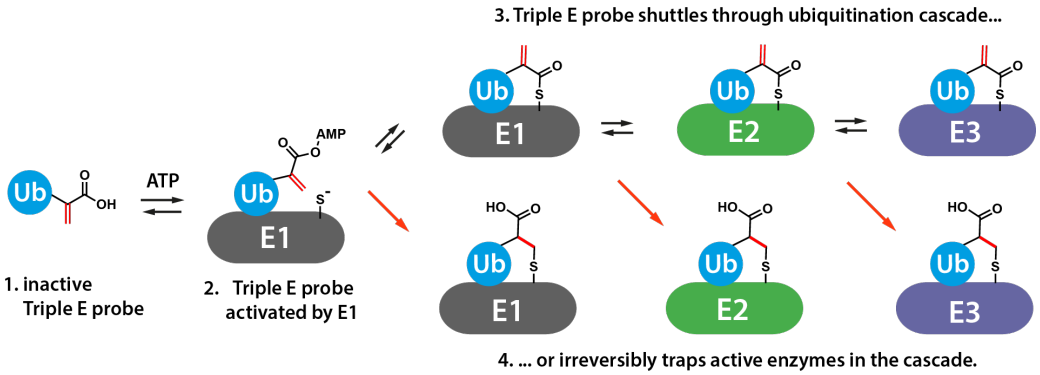

Figure 1. A: sequence. B: mode of action Ub-Dha activity based probes..

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/

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

UbiQ Bio BV
Chamber of Commerce 50023438
VAT NL822502136B01

Science Park 301
1098 XH Amsterdam
The Netherlands

t +31 20 303 1970
e info@ubiqbio.com
i www.ubiqbio.com

Rabobank
IBAN: NL86 RABO 0150658907
BIC/SWIFT: RABONL2U

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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 (labeling can be enhanced by addition of 1-5 mM DTT). The reaction was quenched by the addition of reducing sample buffer and heating (90°C for 10 min).

General Experimental Conditions E1+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 (labeling can be enhanced by addition of 0.5 - 1 mM DTT). The reaction was quenched by the addition of reducing sample buffer and heating (90°C for 10 min).

General Experimental Conditions E1+E2+(HECT/RBR) 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 (labeling can be enhanced by addition of 0.5 - 1 mM DTT). The reaction was quenched by the addition of reducing sample buffer and heating (90°C for 10 min).

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.

Cell lysate labeling.

Cell lysates are resuspended in 3 pellet volumes of HR buffer (50 mM TRIS, pH 7.4, 5 mM $MgCl_2$, 250 mM sucrose, 1 mM DTT) and lysed by sonication. After clarification by centrifugation (20,000 rpm, 4°C, 20 min), total protein concentration is determined by Nanodrop. For labeling experiments, 100 µg of lysate is incubated with 0.5 µg probe, 10 mM ATP, 10 mM $MgCl_2$, in Labeling Buffer (50 mM HEPES, 100 mM NaCl, pH 7.5) at 37°C for 1h or as indicated. Additional 1 mM ATP and $MgCl_2$ were added to the reaction every 20 minutes to replenish consumed ATP. In case of a negative control, lysates were treated with 2 Units of Apyrase (Sigma Aldrich) prior to addition of the probe (ATP and $MgCl_2$ were omitted). The reaction was terminated by the addition of 3x SDS-PAGE Loading Buffer (Invitrogen) containing beta-mercaptoethanol.

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