

UbiQ

targeting the ubiquitin system

Biotin-Ahx-Ub-Dha (human sequence, synthetic)

UbiQ code : UbiQ-102
Batch # : B01042016-001
Amount : 50 ug, lyophilized powder
Purity : ≥95%
Mol. Weight : 8.95 kDa
Storage : upon arrival, powder at –20°C; solution at –80°C. Please avoid multiple freeze/thaw cycles.

Productsheet

Background. UbiQ-102 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 It is based on ubiquitin (Ub) in which Gly76 has been replaced by a dehydroalanine (Dha) residue.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 biotin and a 6-aminohexanoic acid (Ahx) linker is used to create extra space between the biotin and Ub protein for efficient access of biotin binding entities. It has been prepared by total chemical synthesis and is therefore well-defined in terms of biotinylation site. UbiQ-102 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

Biotin-Ahx-MQIFVKTLTGKTTITLEVEPSDTIENVKAKIQDKEGIPPDQQRLLIFAGKQLEDGRTLSDYNIQKESTLHLVLRRLRG-Dha

B

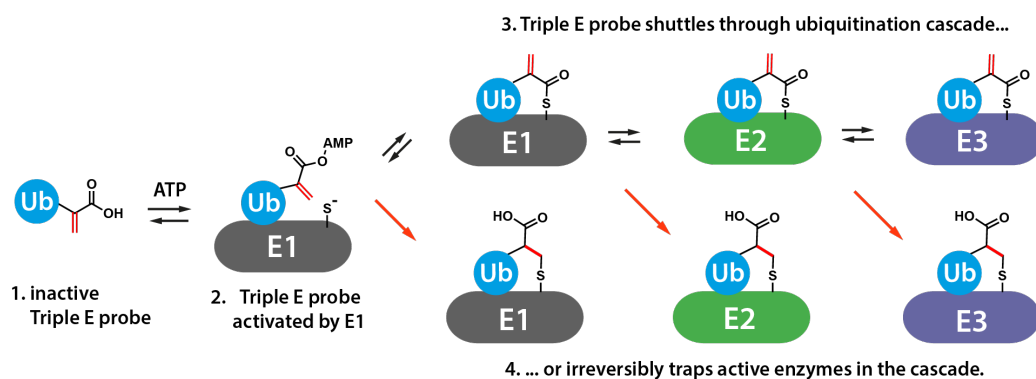


Figure 1. A: sequence. B: mode of action Ub-Dha activity based probes..

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.

General Experimental Conditions E1 labeling assay.

UBE1 or UBA6 (1 μ M) in 50 mM HEPES pH 8, 100 mM NaCl, 10 mM MgCl₂ and 250 μ M ATP was incubated with probe (30 μ M) 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 μ M) and UBE1 (0.63 μ M) in 50 mM HEPES pH 7.5, 100 mM NaCl, 5 mM MgCl₂ and 250 μ M ATP were incubated with probe (12.5 μ M) 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 μ M), UBE2D (0.5 μ M) and UBE1 (0.25 μ M) were incubated with probe (50 μ M) in 50 mM HEPES pH 7.5, 100 mM NaCl, 5 mM MgCl₂ and 250 μ M 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 μ M 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 MgCl₂, 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 μ g of lysate is incubated with 0.5 μ g probe, 10 mM ATP, 10 mM MgCl₂, 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 MgCl₂ 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 MgCl₂ were omitted). The reaction was terminated by the addition of 3x SDS-PAGE Loading Buffer (Invitrogen) containing beta-mercaptoethanol. Samples can be resolved by SDS-PAGE and visualized by biotin antibodies.