UbiQ
targeting the ubiquitin system

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

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

# Productsheet

**Background.** UbiQ-102 is a new and first of its kind activity based probe for Ub E1, E2 and (HECT/RBR) E3 ligases.[1] It is based on the Ub sequence in which the C-terminal Gly76 has been replaced by a dehydroalanine (Dha) residue. The *N*-terminus is labeled with biotin; an 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).

## Sequence

**Biotin-Ahx-MQIFVKTLTGKTITLEVEPSDTIENVKAKIQDKEGIPPDQQRLIFAGKQLEDGRTLSDYNIQKESTLHLVLRLRG-Dha**

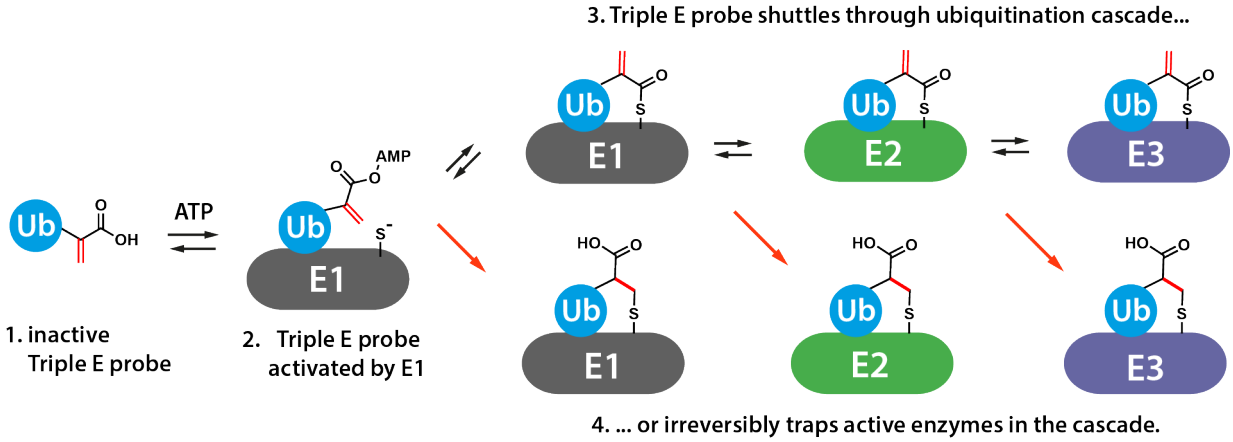


**Figure 1 - Mode of action** of Ub-Dha activity based probes for E1-E2 and (HECT/RBR)-E3 enzymes.

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## 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; this is however not required as in general <5 vol% DMSO is well tolerated by most enzymes.

**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 (90°C for 10 min).

**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).

**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).

**Literature.** (1) (a) Mulder et al. *Nat. Chem. Biol.* **2016**, doi DOI: 10.1038/NCHEMBIO.2084. (b) MPC Mulder, F. El Oualid and H. Ovaa. Adenylation enzyme inhibitors. Application WO/2016/032332 and NL2015/050596

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