

Nedd8-Dha (*human Nedd8 sequence, synthetic*)

UbiQ code : UbiQ-105
 Batch # : B01072015-001
 Amount : 50 ug, lyophilized powder
 Purity : ≥95% by RP-HPLC
 Mol. Weight : 8.57 kDa
 Storage : powder at -20°C; solution at -80°C. Please avoid multiple freeze/thaw cycles.

Productsheet

Background. UbiQ-105 is a new and first of its kind activity based probe for Nedd8 E1, E2 and E3 ligases.¹ It is based on the Nedd8 sequence in which the C-terminal Gly76 has been replaced by a dehydroalanine (Dha) residue. UbiQ-105 is processed in a native manner by E1, E2 and 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 E3 enzyme, thereby creating a covalent bond (Figure 1).

Sequence

MLIKVKLTG KEIEIDIEPT DKVERIKERV EEKEGIPPQQ QRLIYSGKQM NDEKTAADYKILGGSVLHLV LALRG -Dha

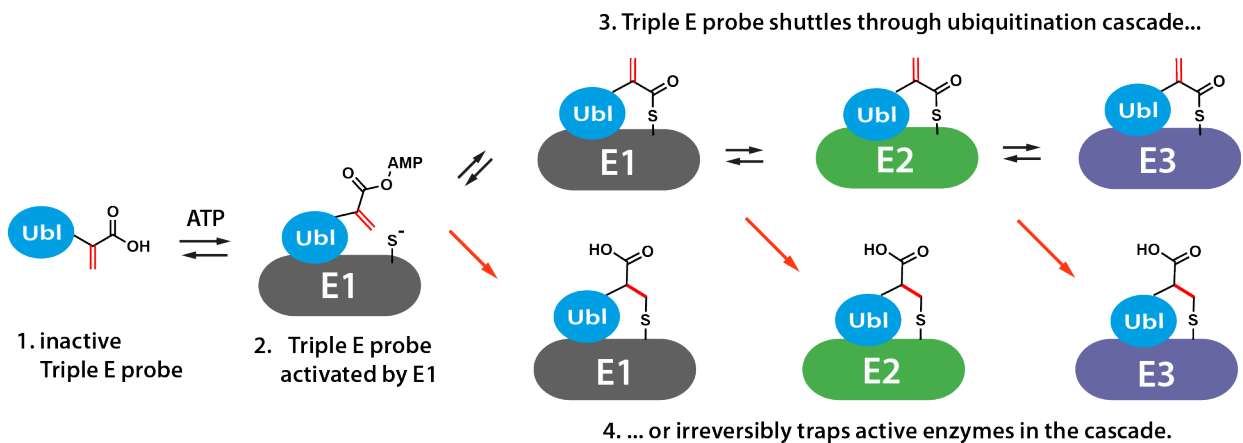


Figure 1 - Mode of action of Ubi-Dha activity based probes for E1-E2-E3 enzymes.

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.

E1 (1 μM) in 50 mM HEPES pH 8, 100 mM NaCl, 10 mM MgCl_2 and 250 μM 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 E1 (0.63 μM) in 50 mM HEPES pH 7.5, 100 mM NaCl, 5 mM MgCl_2 and 250 μM 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.

E3 (2.5 μM), E2 (0.5 μM) and E1 (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 μM 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