UbiQ
targeting the ubiquitin system

Nedd8-Dha *(human sequence, synthetic)*

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

Productsheet

Background. UbiQ-105 is an activity-based probe for Nedd8 E1, E2 and (HECT/RBR type) E3 ligases. It is based on Nedd8 in which Gly76 has been replaced by a dehydroalanine (Dha) residue. It is processed in a native manner by Nedd8 E1, E2 and (HECT/RBR) E3 enzymes 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 300 mM NaCl (please note the order of addition) and mix - *at this step we have included a high salt aq. solution because Nedd8 is more stable at high salt concentration.*
- 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.
E1 (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 E1 (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.
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 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).

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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A

MLIKVKTLTGKEIEIDIEPTDKVERIKERVEEKEGIPPQQQRLIYSGKQMNDEKTAADYKILGGSVLHLVLALRG-Dha

B

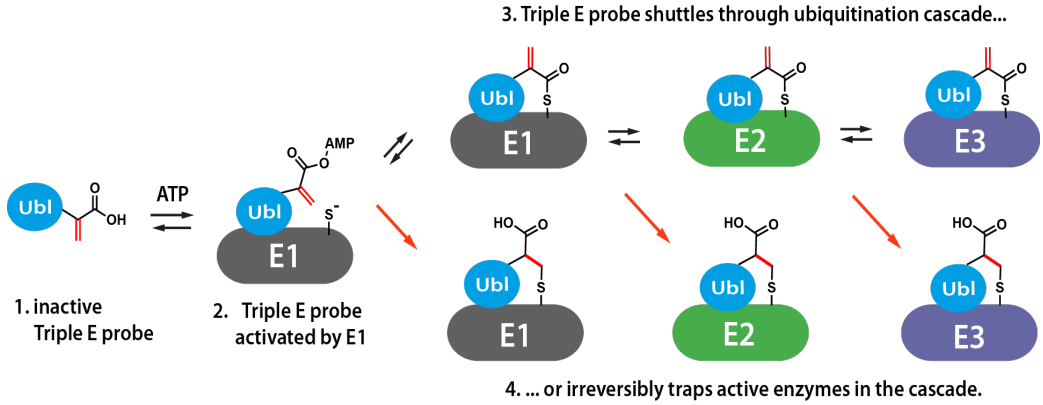

Figure 1. A: sequence. B: D: Mode of action Nedd8-Dha activity-based probes.

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