

UbiQ

targeting the ubiquitin system

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

UbiQ code : UbiQ-106
Batch # : B01072015-001
Amount : 50 ug, lyophilized powder
Purity : ≥95%
Mol. Weight : 8.93 kDa
Storage : upon arrival, powder at -20°C; solution at -80°C. Please avoid multiple freeze/thaw cycles.

Productsheet

Background. UbiQ-106 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 It is based on Nedd8 in which Gly76 has been replaced by a dehydroalanine (Dha) residue. The N-terminus is labeled with biotin; a 6-aminohexanoic acid (Ahx) linker is used to create extra space between the biotin and Nedd8 protein for efficient access of biotin binding entities. UbiQ-106 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).

important: sample preparation

- dissolve the powder in as little DMSO as possible (e.g. 40 mg/mL)
- add this DMSO stock slowly to water; *we advise to use 300 mM NaCl as Nedd8 shows enhanced stability at high salt concentrations (please note to add DMSO to this aq solution).*
- 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 E1 labeling conditions

- UBA3/NAE1 (2 μM) is incubated with 40 μM probe in 50 mM Tris pH 7.5, 300 mM NaCl, 10 mM MgCl₂ and (0.25 - 5 mM) ATP at 30°C for 90 min.
- for SDS-PAGE analysis, the reaction is quenched by the addition of reducing sample buffer and heated at 90°C for 10 min.

General E1-E2 labeling conditions

- UBE2M (2 μM) is incubated with UBA3/NAE1 (0.5 μM) and 20 μM probe in buffer containing 50 mM Tris pH 7.5, 300 mM NaCl, 10 mM MgCl₂ and (0.25 - 5 mM) ATP at 30°C for 60 min.

Please note optimal reaction conditions can vary between E2 and E3 enzymes.

- it is advised to vary the ATP concentration from 250 μM 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

Biotin-Ahx-MLIKVKTLTGKEIEIDIEPTDKVERIKERVEEKEGIPPQQQLIYSGKQMNDEKTAADYKILGGSVLHLVLALRG-Dha

B

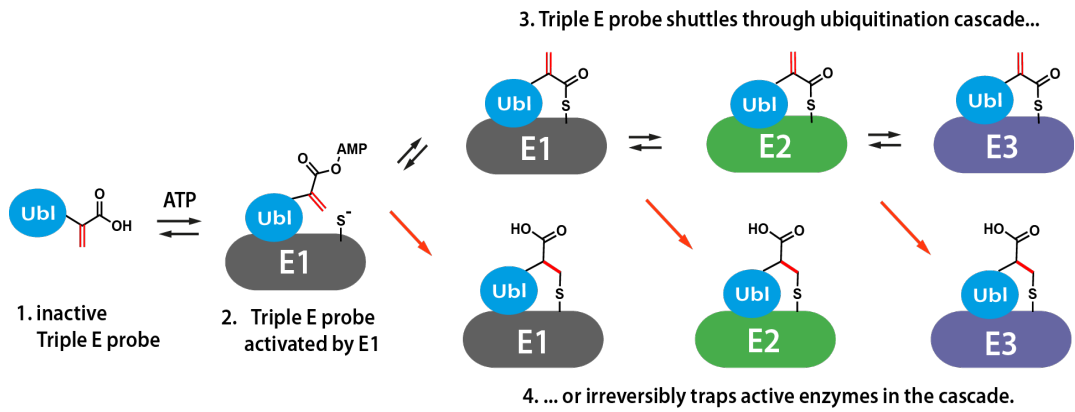


Figure 1. A: sequence. B: D: Mode of action Nedd8-Dha activity-based probes.