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% by RP-HPLC
Mol. Weight	:	8.93 kDa
Storage	:	powder at –20°C; solution at –80°C. Please avoid multiple freeze/thaw cycles.

Productsheet

Background. UbiQ-106 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based probe for Nedd8 E1, E2 and E3 ligases.[1] It is based on the Nedd8 sequence in which the C-terminal Gly76 has been replaced by a dehydroalanine (Dha) residue. The *N*-terminus is labeled with biotin; an aminohexanoic acid (Ahx) linker is used to create extra space between the biotin and Nedd8 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-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).

Sequence

Biotin-Ahx-
MLIKVKTLTGKEIEIDIEPTDKVERIKERVEEKEGIPPQQQRLIYSGKQMNDEKTAADYKILGGSVLHLVLALRG-Dha

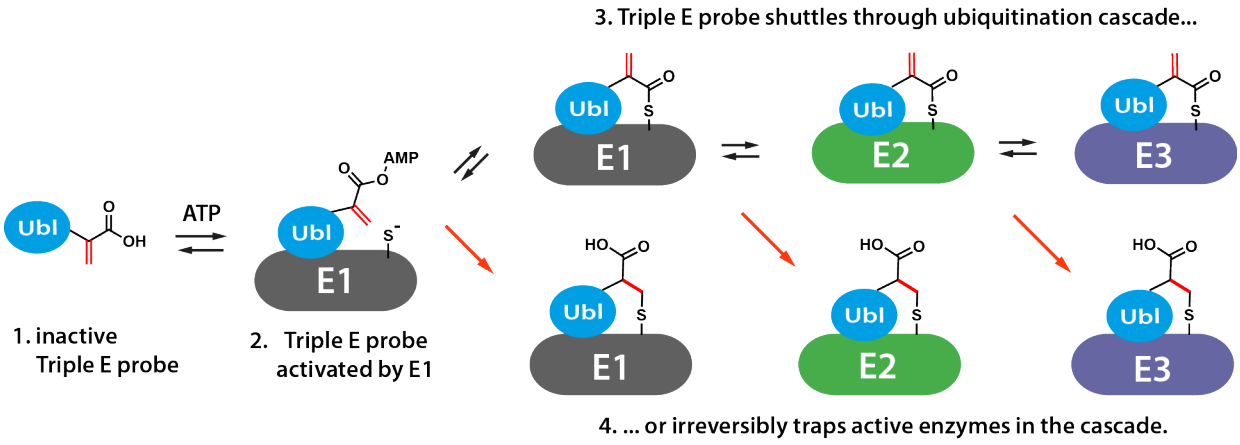

Figure 1 - Mode of action of Ubl-Dha activity based probes for E1-E2-E3 enzymes.

UbiQ
targeting the ubiquitin system

Important: sample preparation

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

- UBA3/NAE1 (2 µM) is incubated with UbiQ-106 (40 µM) in 50 mM Tris pH 7.5, 300 mM NaCl, 10 mM $MgCl_2$ 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 UbiQ-106 (20 µM) in buffer containing 50 mM Tris pH 7.5, 300 mM NaCl, 10 mM $MgCl_2$ and (0.25 - 5 mM) ATP at 30°C for 60 min.

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

- **I**t is advised to vary the ATP concentration from 250 uM to 5 mM and determine which is best for your experiment.

Literature. (1) (a) Mulder et al. Mulder et al. *Nat. Chem. Biol.* **2016**, *12*, 523. (b) MPC Mulder, F. El Oualid and H. Ovaa. Adenylation enzyme inhibitors. Application WO/2016/032332 and NL2015/050596

UbiQ Bio BV
Chamber of Commerce 50023438
VAT NL822502136B01

Science Park 408
1098 XH Amsterdam
The Netherlands

t +31 20 303 1970
e info@ubiqbio.com
i www.ubiqbio.com

Rabobank
IBAN NL86RABO0150658907
BIC/SWIFT RABONL2U