UbiQ
targeting the ubiquitin system

5-carboxyRh110-Nedd8-Dha *(human Nedd8 sequence, synthetic)*

UbiQ code	:	UbiQ-122
Batch #	:	B01072015-001
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-122 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based probe for Nedd8 E1, E2 and E3 ligases.[1] It is based on the Nedd8 sequence in which the C-terminal Gly76 has been replaced by a dehydroalanine (Dha) residue.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 the green fluorescent 5-carboxyrhodamine110 dye (cRh110). It has been prepared by total chemical synthesis and is therefore well-defined in terms of dye site. UbiQ-122 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

cRh110-MLIKVKTLTG KEIEIDIEPT DKVERIKERV EEKEGIPPQQ QRLIYSGKQM NDEKTAADYKILGGSVLHLV LALRG-Dha

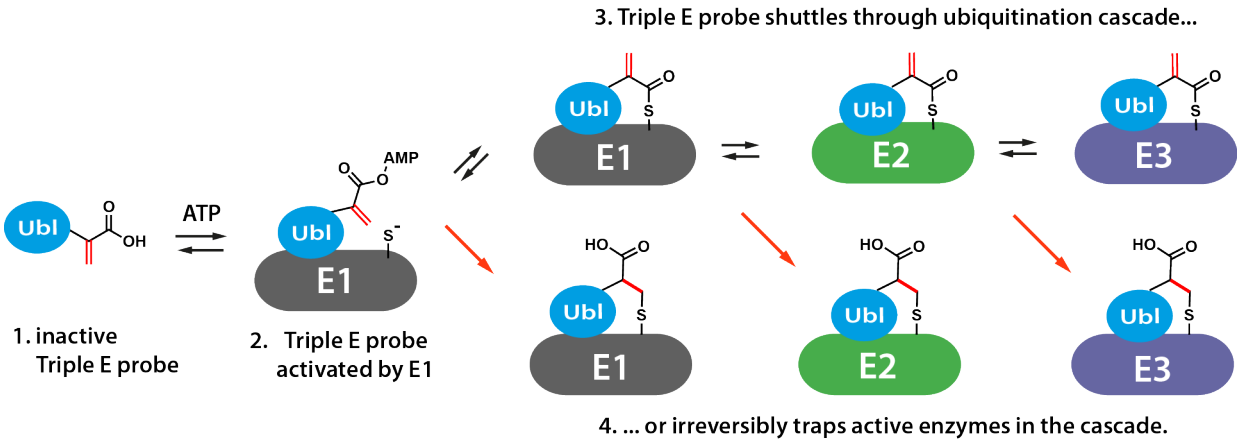

Figure 1 - Mode of action of Ubl-Dha activity based probes for E1-E2-E3 enzymes.

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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.
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).

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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