UbiQ

targeting the ubiquitin system

Cy5-Ub-Dha

UbiQ code : UbiQ-104
Batch # : B01072015-001
Amount : 50 ug, lyophilized powder
Purity : ≥95% by RP-HPLC
Mol. Weight : 9.05 kDa
Storage : powder at –20°C; solution at –80°C. Please avoid multiple freeze/thaw cycles.

Productsheet

Background. UbiQ-104 is a new and first of its kind activity based probe for Ub E1, E2 and (HECT/RBR) E3 ligases.[1] It is based on the Ub sequence in which the C-terminal Gly76 has been replaced by a dehydroalanine (Dha) residue. The *N*-terminus is labeled with a Cy5 tag (λex = 625 nm; λem = 680 nm). It has been prepared by total chemical synthesis and is therefore well-defined in terms of Cy5 site.

UbiQ-104 is processed in a native manner by Ub E1, E2 and (HECT/RBR) 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 (HECT/RBR) E3 enzyme, thereby creating a covalent bond (Figure 1).

Sequence

Cy5-MQIFVKTLTGKTITLEVEPSDTIENVKAKIQDKEGIPPDQQRLIFAGKQLEDGRTLSDYNIQKESTLHLVLRLRG-Dha

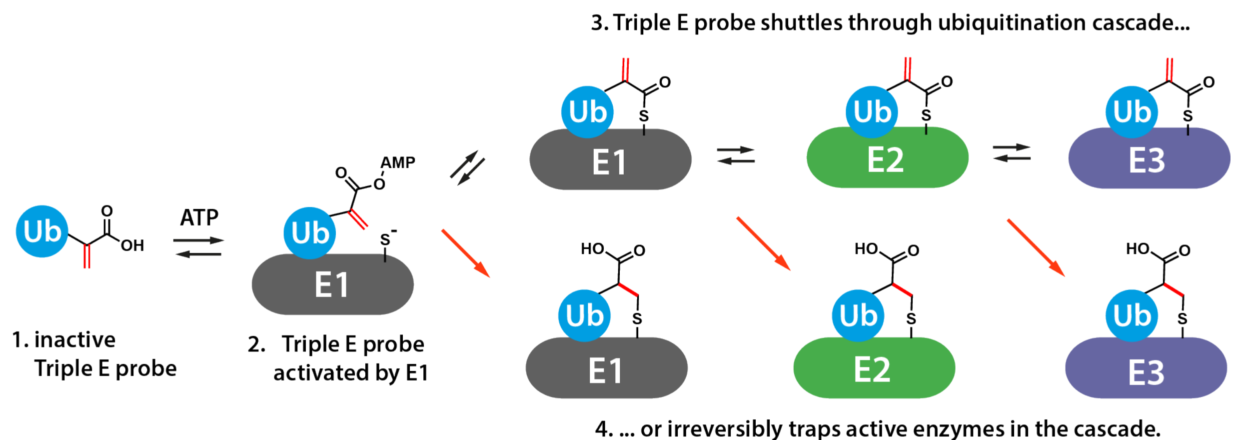

Figure 1 - Mode of action of Ub-Dha activity based probes for E1-E2 and (HECT/RBR)-E3 enzymes.

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Important: sample preparation

- **dissolve the powder in as little DMSO as possible (e.g. 20 mg/mL)**
- **add this DMSO stock slowly to milliQ (please note the order of addition)**
- **buffer the aq. solution as desired (e.g. 50 mM HEPES pH 8, 100 mM NaCl)**
- **final stocks of e.g. 0.5 mg/mL will contain 2.5 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.
UBE1 or UBA6 (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 E1+E2 labeling assay.
E2 enzyme (2.5 µM) and UBE1 (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 E1+E2+(HECT/RBR) E3 labeling assay.
Nedd4L (2.5 µM), UBE2D (0.5 µM) and UBE1 (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).

Cell lysate labeling.
Cell lysates are resuspended in 3 pellet volumes of HR buffer (50 mM TRIS, pH 7.4, 5 mM $MgCl_2$, 250 mM sucrose, 1 mM DTT) and lysed by sonication. After clarification by centrifugation (20,000 rpm, 4°C, 20 min), total protein concentration is determined by Nanodrop. For labeling experiments, 100 µg of lysate is incubated with 0.5 µg **Cy5-Ub-Dha**, 10 mM ATP, 10 mM $MgCl_2$, in Labeling Buffer (50 mM HEPES, 100 mM NaCl, pH 7.5) at 37°C for 1h or as indicated. Additional 1 mM ATP and $MgCl_2$ were added to the reaction every 20 minutes to replenish consumed ATP. In case of a negative control, lysates were treated with 2 Units of Apyrase (Sigma Aldrich) prior to addition of the probe (ATP and $MgCl_2$ were omitted). The reaction was terminated by the addition of 3x SDS-PAGE Loading Buffer (Invitrogen) containing beta-mercaptoethanol. Samples were resolved by SDS-PAGE and visualized by fluorescence scanning (λex = 625 nm; λem = 680 nm).

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