UbiQ
targeting the ubiquitin system

Ub-Dha

UbiQ code	:	UbiQ-101
Batch #	:	B01042015-001
Amount	:	50 ug, lyophilized powder
Purity	:	≥95% by RP-HPLC
Mol. Weight	:	calc 8577 Da, found 8575 Da
Storage	:	powder at −20°C; solution at −80°C. Please avoid multiple freeze/thaw cycles.

Productsheet

Background. UbiQ-101 (Ub-Dha, Dha= dehydroalanine) is a new and first of its kind activity based probe for Ub E1, E2 and (HECT/RBR) E3 ligases. It is prepared by total chemical synthesis and based on the Ub sequence in which the C-terminal Gly76 has been replaced by a dehydroalanine residue. It 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 (Fig. 2).

Sequence

MQIFVKTLTGKTITLEVEPSDTIENVKAKIQDKEGIPPDQQRLIFAGKQLEDGRTLSDYNIQKESTLHLVLRLRG-**Dha**

Important: sample preparation

- **dissolve the powder in as little DMSO as possible (e.g. 20 mg/mL, if desired one can make a more concentrated DMSO stock of e.g. 40 mg/mL)**
- **add the 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)**
- **for example, a final buffered stock of UbiQ-101 of 0.5 mg/mL (57 uM) will contain 2.5 vol% DMSO when prepared from a 20 mg/mL DMSO stock.**
- **in general, E1-E2 and E3 enzymes tolerate DMSO concentrations up to 5 vol%**
- **if desired, the DMSO can be removed from the buffered stock by dialysis or 3 kDa spin-filters**

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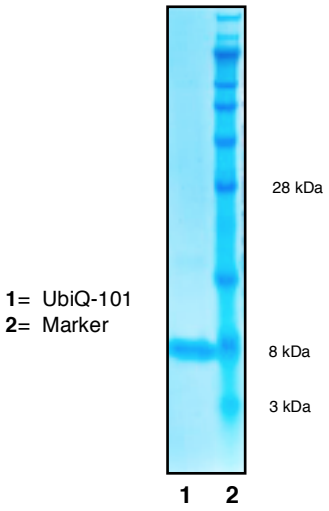
A



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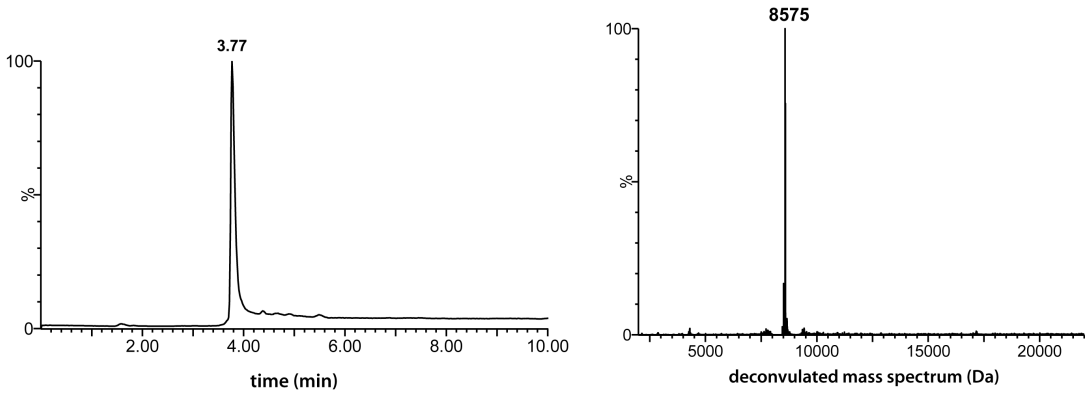

Figure 1. A: SDS-PAGE analysis UbiQ-101 (B01042015-001). 12% Bolt Bis-Tris Plus gel (Life technologies) and MES running buffer. Marker= SeeBlue Plus2 Pre-stained Standard (Invitrogen). CBB staining was performed with a Coommassie G-250 solution. **B: LC-MS analysis** UbiQ-101 (B01042015-001)**.** Mobile phase A= 1% CH_3CN, 0.1% formic acid in milliQ and B= 1% milliQ and 0.1% formic acid in CH_3CN. XBridge BEH300 C18 5µm 4.6x100mm; column T= 40°C, flow= 0.8 mL/min. Gradient: 30–60%B over 6.5 min.

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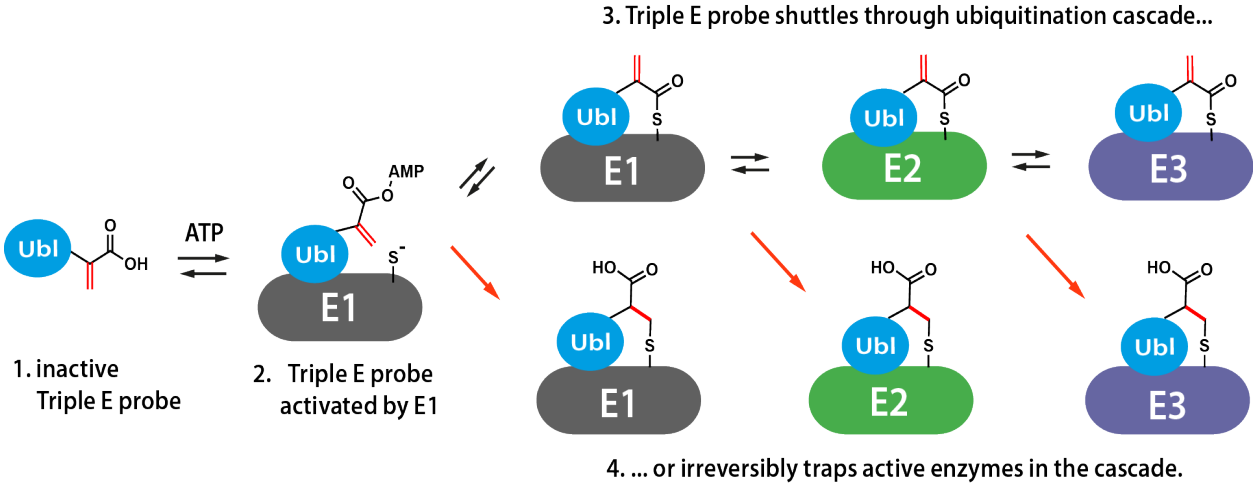

Figure 2. Mode of action of Ub-Dha activity based probes for E1-E2 and (HECT/RBR)-E3 enzymes.

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). Samples can be analyzed by SDS-PAGE using Coomassie staining.

General Experimental Conditions 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). Samples can be analyzed by SDS-PAGE using Coomassie staining.

General Experimental Conditions HECT 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). Samples can be analyzed by SDS-PAGE using Coomassie staining.

For more detailed experimental background, please see reference 1.

Literature. (1) Mulder et al. *Nat. Chem. Biol.* **2016**, *12*, 523. (2) M.P.C. Mulder, F. El Oualid and H. Ovaa. Adenylation enzyme inhibitors. Application WO/2016/032332 and NL2015/050596