

Biotin-Ahx-SUMO2-Dha (human sequence, synthetic)

UbiQ code : UbiQ-159
 Batch # : B01042016-001
 Amount : 50 ug, lyophilized powder
 Purity : ≥95% by RP-HPLC
 Mol. Weight : 10.94 kDa
 Storage : powder at -20°C; solution at -80°C. Please avoid multiple freeze/thaw cycles.

Productsheet

Background. UbiQ-159 (Dha= dehydroalanine) is a mechanism activity-based probe for the SUMO ligation pathway.¹ It is based on the SUMO2 sequence in which the C-terminal Gly has been replaced by Dha. The N-terminus is labeled with biotin and an aminohexanoic acid (Ahx) linker is used to create extra space between the biotin and Ub 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-159 is processed in a native manner by SUMO E1, E2 and E3 ligases and during this process it forms an electrophilic intermediate that can react with catalytic active site Cys residues, thereby creating a covalent bond (Fig. 1).

Sequence

Biotin-Ahx-

MADEKPKEGVKTENNDHINLKVAGQDGSVVQFKIKRHTPLSKLMKAYSERQGLSMRQIRFRFDGQPINETDTPAQLEMEDEDTID
 VFQQQTG-Dha

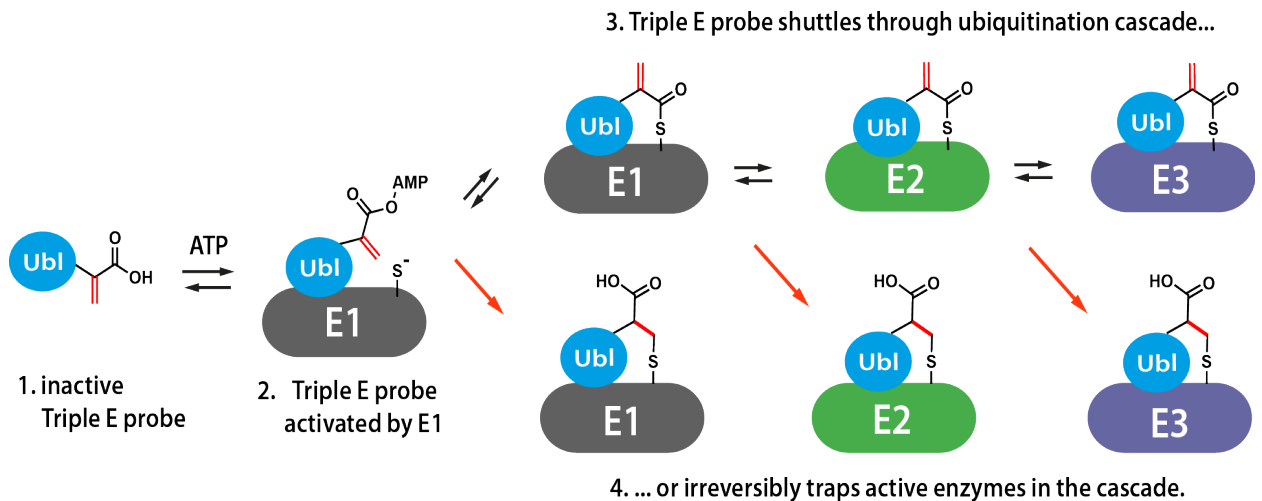
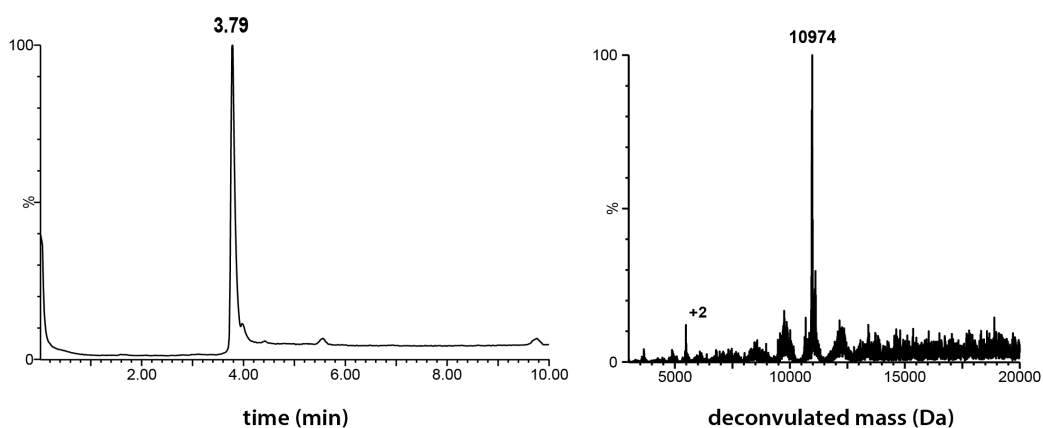


Figure 1. Mode of action of Dha activity-based probes for E1-E2 and E3 enzymes.

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.



LC-MS analysis. Mobile phase A = 1% CH₃CN, 0.1% formic acid in water (milliQ) and B = 1% water (milliQ) and 0.1% formic acid in CH₃CN. XBridge BEH300 C18 5 μ m 4.6x100mm; column T = 40°C, flow= 0.8 mL/min. Gradient: 30–60% over 6.5 min.

Literature. (1) (a) 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