

Biotin-Ahx-Ub(1-75)-Dha-Ub (human sequence, synthetic)

UbiQ code : UbiQ-121
Batch # : B01062015-001
Amount : 50 ug, lyophilized powder
Purity : ≥95% by SDS-PAGE analysis
Mol. Weight : 17.47 kDa
Storage : upon arrival powder at –20°C, solution at –80°C. Avoid multiple freeze/thaw cycles.

Productsheet

Background. UbiQ-121 is an activity based probe based on linear diubiquitin which targets the deubiquitinating enzyme (DUB) OTULIN.^{1,*} In UbiQ-121, the Gly76-Met1 dipeptide of linear diUb is replaced by a dehydroalanine-Met (**Dha-Met**) dipeptide (Figure 1). The *N*-terminus of the distal Ub is labeled with biotin; an aminohexanoic acid (Ahx) linker is used to create extra space for efficient access of biotin binding entities. UbiQ-121 has been prepared by total chemical synthesis and is therefore well-defined in terms of biotinylation site. UbiQ-121 reacts with OTULIN in a covalent manner (data not shown) without being cleaved into monoUb, indicating correct positioning of the Dha electrophile for reacting with the nucleophilic active site cysteine of OTULIN. The DUB activity based probe can be used for activity profiling experiments and structural studies.¹⁻¹⁰ Please note that the native distance between the proximal and distal Ub is preserved.

* Please note that, due to Gly76 of the proximal Ub, UbiQ-121 shows some cross-reactivity with USP5 (isoT) and can serve as substrate for Ub E1-E2-E3 enzymes (resulting in the probe being incorporated into chains).

Sequence

Biotin-Ahx-MQIFVKLTGKTITLEVEPSDTIENVKAKIQDKEGIPPDQQLIFAGKQLEDGRTLSDYNIQKESTLHLVLRRLRG-
Dha-Met-QIFVKLTGKTITLEVEPSDTIENVKAKIQDKEGIPPDQQLIFAGKQLEDGRTLSDYNIQKESTLHLVLRRLGG

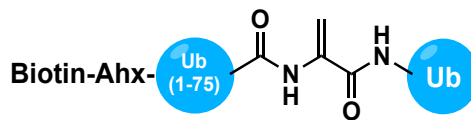
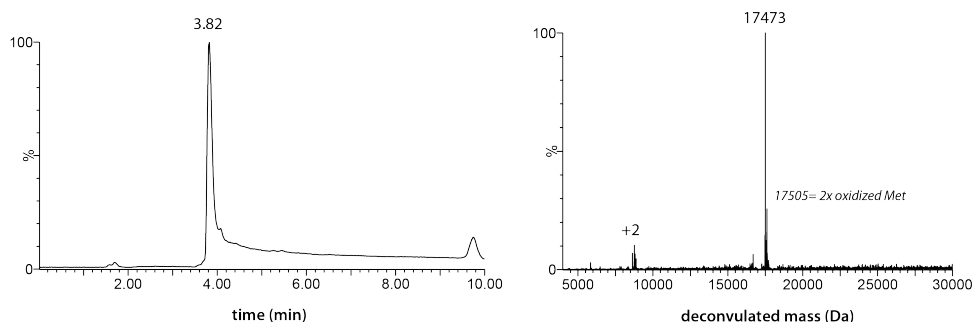


Figure 1. UbiQ-121

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
- 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.
- for full experimental details see open-access reference 1: <http://dx.doi.org/10.1016/j.chembiol.2017.08.006>



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

Literature. (1) Weber et al. *Cell Chem Biol.* **2017**, DOI: <http://dx.doi.org/10.1016/j.chembiol.2017.08.006> (2) Mulder et al. *ChemBioChem* **2014**, *15*, 946. (3) Misaghi et al. *J. Biol. Chem.* **2005**, *280*, 1512. (4) de Jong et al. *ChemBioChem* **2012**, *13*, 2251. (5) Altun et al. *Chem. Biol.* **2011**, *18*, 1401. (6) Haj-Yahya et al. *Org. Lett.*, **2014**, *16*, 540. (7) Li et al. *Chem. Commun.* **2014**, *50*, 216. (8) Iphöfer et al. *ChemBioChem* **2012**, *13*, 1416. (9) McGouran et al. *Chem. Biol.* **2013**, *20*, 1447. (10) Haj-Yahya et al. *Org. Lett.* **2014**, *16*, 540.