

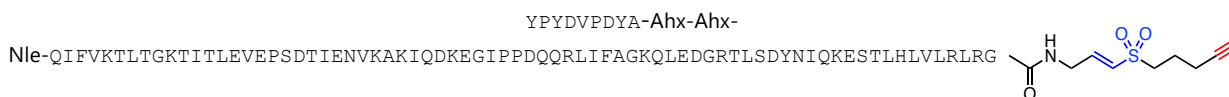
## HA-Ahx-Ahx-Ub-VPS (*VS-alkyne*= vinyl pentynyl sulfone= VPS, human sequence, Met1Nle, synthetic)

UbiQ code : UbiQ-193  
 Batch # : B01112016-001  
 Amount : 50 ug, lyophilized powder  
 Purity : ≥95% by RP-HPLC  
 Mol. Weight: 9.97 kDa  
 Storage : upon arrival, powder at –20°C; solution at –80°C. Avoid multiple freeze/thaw cycles.

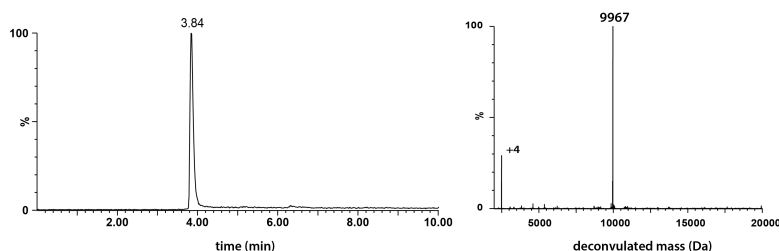
## Productsheet

**Background.** UbiQ-193 is an activity-based probe for deubiquitinating enzymes (DUBs). It contains a vinyl pentynyl sulfone (VPS) warhead (Figure 1, alternative name VS-alkyne). UbiQ-193 contains an N-terminal HA-tag (YPYDVPDYA), which is a peptide sequence derived from the influenza hemagglutinin protein and allows for the sensitive identification or purification by anti-HA antibodies and/or anti-HA-agarose. The HA tag is separated from the Ub N-terminus by two aminohexanoic acid (Ahx) linkers for efficient recognition of the tag. To eliminate Met1 oxidation, Met1 is replaced by norleucine, a well validated Met mimic.

**A**



**B**



**Figure 1.** A: sequence. B: LC-MS analysis. Mobile phase A= 1% CH<sub>3</sub>CN, 0.1% formic acid in water and B= 1% water and 0.1% formic acid in CH<sub>3</sub>CN. XBridge BEH300 C18 5µm 4.6x100mm; column T = 40°C, flow= 0.8 mL/min. Gradient: 30–60%B over 6.5 min.

### important: sample preparation

- dissolve the powder in as little DMSO as possible (e.g. 20 mg/mL) and add this DMSO stock slowly to milliQ (please note the order of addition); mix by vortex.
- next buffer as desired. For example:
  - 50 ug probe in 2.5 uL DMSO (20 mg/mL, 2 mM)
  - example 1: add to 47 uL water followed by addition of 0.5 uL 5M NaOAc pH 4.5 to prepare a 1 mg/mL stock in 50 mM NaOAc pH 4.5 (100 uM); this stock is useful when working with low concentrations of probe
  - example 2: add to 45 uL water followed by addition of e.g. 2.5 uL 1M HEPES to prepare a 1 mg/mL stock in 50 mM HEPES (100 uM); this stock is useful when working with high concentrations of probe
- full experimental details can be found here: <https://www.ncbi.nlm.nih.gov/pubmed/29563501>
- please be aware of background bands due to cross-reactivity of anti-HA antibodies

Please note we and others have observed the appearance of smearing during SDS-PAGE analysis of (di)Ub conjugates. This can be caused by (heat-induced) aggregation (Morimoto et al. Sci Rep 2018, 8, article 2711). If possible, avoid heating the samples in Laemmli sample buffer for SDS-PAGE analysis and/or add 4M urea to the SDS-PAGE samples.

**Literature.** (1) El Oualid et al. *Angew. Chem. Int. Ed.* **2010**, 49, 10149. (2) Hewings et al. *Nat. Commun.* **2018**, 9, article number 1162. (3) Xu et al. *RSC Adv* **2016**, 6, 47926.