UbiQ
targeting the ubiquitin system

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	:	9.96 kDa
Storage	:	upon arrival, powder at –20°C; solution at –80°C. Avoid multiple freeze/thaw cycles.

Productsheet

Background. UbiQ-193 is an irreversible inhibitor of deubiquitinating enzymes (DUBs)[1] that is prepared by chemical synthesis.[2] It contains a vinyl pentynyl sulfone (VPS) warhead (Figure 1, *alternative name VS-alkyne*).[3] 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.[1] 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.[4]

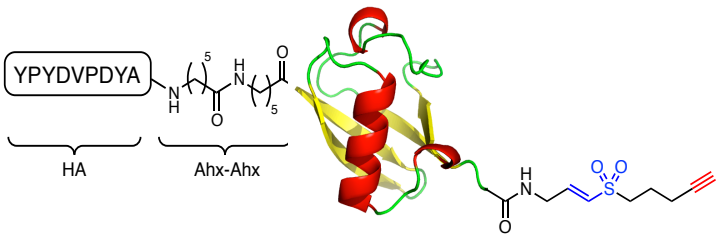
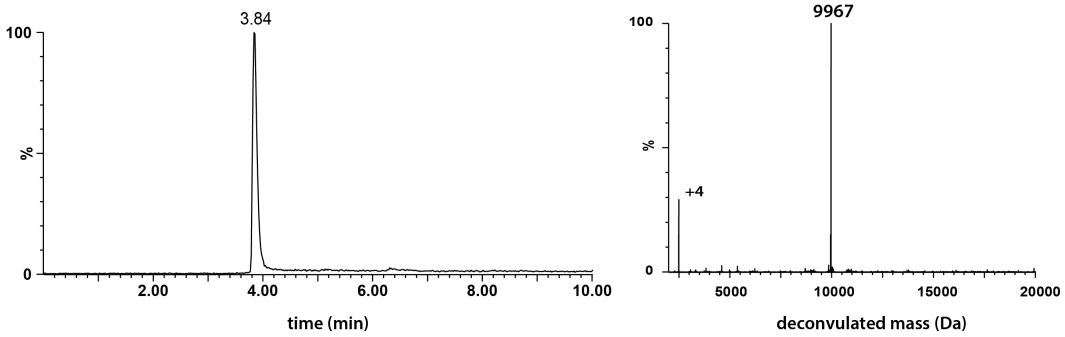


Figure 1



LC-MS analysis. Mobile phase A = 1% CH_3CN, 0.1% formic acid in water (milliQ) and B = 1% water (milliQ) and 0.1% formic acid in CH_3CN. XBridge BEH300 C18 5µm 4.6x100mm; flow rate = 0.8 mL/min, runtime = 10 min, column T = 40°C. Gradient: 30% ⇨ 60% B over 6.5 min.

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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 2.5 uL 1M HEPES or Tris to prepare a 1 mg/mL stock in 50 mM HEPES/Tris (100 uM); this stock is useful when working with high concentrations of probe
- please note that DUBs can react different to low or high NaCl concentration
- in general, DMSO concentrations of up to 5 vol% are well tolerated by DUBs
- full experimental details can be found here[3]: https://www.ncbi.nlm.nih.gov/pubmed/29563501
- please be aware of background bands due to cross-reactivity of anti-HA antibodies[1a]

Literature. (1) (a) de Jong et al. *ChemBioChem* **2012**, *13*, 2251. (b) Borodovsky et al. *EMBO J.* **2001**, *20*, 5187. (c) Borodovsky et al. *Chem. Biol.* **2002**, *9*, 1149. (2) El Oualid et al. *Angew. Chem. Int. Ed.* **2010**, *49*, 10149. (3) Hewings et al. *Nat. Commun.* **2018**, *9*, article number 1162. (4) Xu et al. *RSC Adv* **2016**, *6*, 47926.

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