

UbiQ

targeting the ubiquitin system

HA-Ahx-Ahx-Ub-VS-alkyne (human sequence, synthetic)

UbiQ code : UbiQ-193
Batch # : B01112016-001
Amount : 1.00 mg, lyophilized powder
Purity : $\geq 95\%$ by RP-HPLC
Mol. Weight : 9.96 kDa
Storage : upon arrival powder at -20°C ; solution at -80°C . Avoid multiple freeze/thaw cycles.

UbiQ-193 was exclusively made for and commissioned by Genentech. In accordance with the SOW dated and effective September 5, 2016. For this work UbiQ incorporated the building block as supplied by Genentech into a probe for deubiquitinase enzyme activity.

Productsheet

Background. **UbiQ-193** is an irreversible inhibitor of deubiquitinating enzymes (DUBs)¹ that is prepared by chemical synthesis.² It contains a vinylsulfone (VS) warhead which is equipped with an alkyne group (Figure 1). 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.³

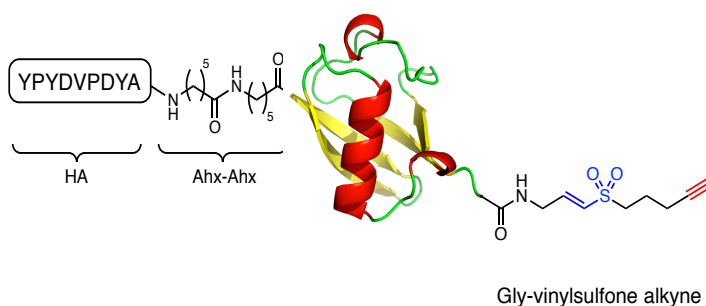
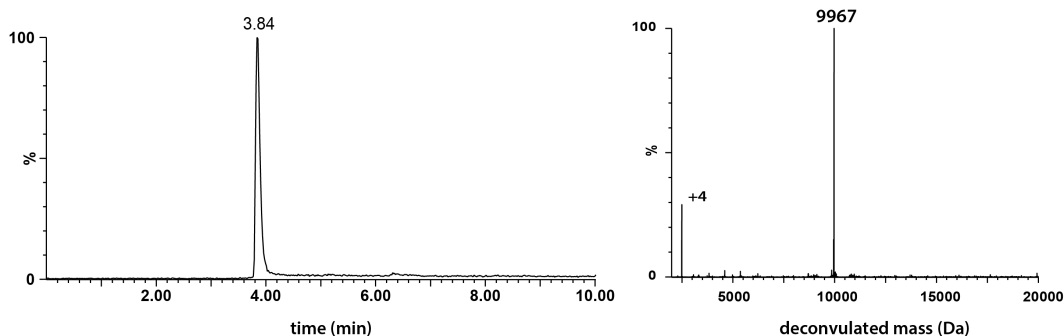


Figure 1

Sequence

YPYDVPDYA-Ahx-Ahx-NleQIFVKTLTGKTITLEVEPSDTIENVKAKIQDKEGIPPDQQLRIFAGKQLEDGRTLSDYNIQKESTLHLVLRIRGG-
vinylsulfone alkyne



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; flow rate = 0.8 mL/min, runtime = 10 min, column T = 40°C. Gradient: 30% \Rightarrow 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).
- next buffer as desired. In general HEPES and Tris buffers are standard for DUB assays. Please note that certain DUBs react different to low or high NaCl concentrations.
- a final buffered stock of for example 0.5 mg/mL contains 2.5 vol% DMSO; in general DMSO concentrations of up to 5 vol% are well tolerated by DUBs.
- if required, total removal of DMSO is accomplished by dialysis or spin-filtration (3 kDa cut-off membrane).
- 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) Xu et al. *RSC Adv* **2016**, *6*, 47926.