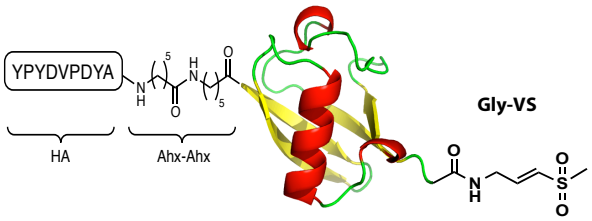
UbiQ
targeting the ubiquitin system



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

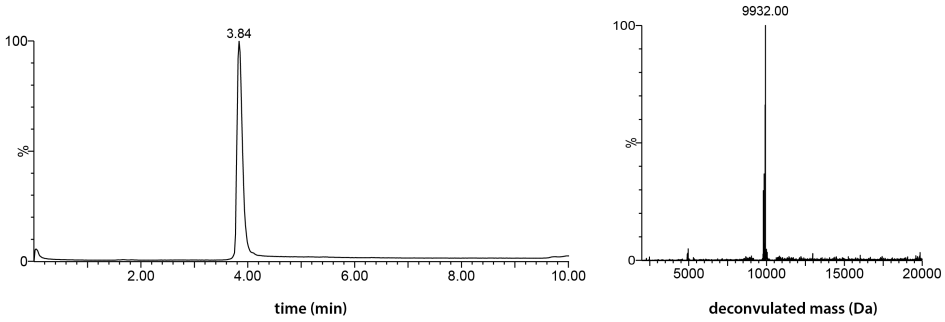
UbiQ code	:	UbiQ-187
Batch #	:	B01112016-001
Amount	:	50 ug, lyophilized powder
Purity	:	≥95% by RP-HPLC
Mol. Weight	:	9.93 kDa
Storage	:	upon arrival powder at –20°C; buffered solution at –80°C. Please avoid multiple freeze/thaw cycles.

Productsheet

Background. HA-Ahx-Ahx-Ub-VS (**UbiQ-187**) is a potent and specific inhibitor of deubiquitinating enzymes (DUBs) based on a *C*-terminal electrophilic vinyl sulfone (VS) group.[1,2] UbiQ-187 can be used for activity profiling experiments and determining DUB inhibitor specificity. UbiQ-187 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.[1] To eliminate Met1 oxidation, Met1 is replaced by norleucine, a well validated Met mimic.[3]

Sequence

YPYDVPDYA-Ahx-Ahx-NIeQIFVKTLTGKTITLEVEPSDTIENVKAKIQDKEGIPPDQQRLIFAGKQLEDGRTLSDYNIQKESTLHLVLRLRGG-**VS**



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.

Important: sample preparation

- **dissolve the powder in as little DMSO as possible (20 - 40 mg/mL)**
- **add the DMSO stock to milliQ (please note the order of addition) and mix**
- **buffer the aq. solution as desired (using 1M HEPES or 1M Tris for example)**
- **in general, DMSO concentrations up to 5 vol% are well tolerated by most enzymes.**
- **If required, total removal of DMSO is accomplished by dialysis or spin-filtration (3 kDa cut-off membrane).**

Literature. (1) de Jong et al. *ChemBioChem* **2012**, *13*, 2251. (2) Borodovsky et al. **2001**, *20*, 5187. (3) Xu et al. *RSC Adv* **2016**, *6*, 47926.

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