UbiQ
targeting the ubiquitin system

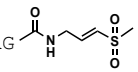
HA-Ahx-Ahx-Ub-VS *(human sequence, synthetic)*

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. UbiQ-187 is an activity-based probe for deubiquitinating enzymes (DUBs). It contains a C-terminal electrophilic vinyl sulfone (VS) group and is labeled on the N-terminus with the HA peptide sequence (YPYDVPDYA) derived from the influenza hemagglutinin protein and allows for the sensitive identification or purification of DUBs by anti-HA antibodies and/or anti-HA-agarose. The HA tag is separated from the N-terminus by two 6-aminohexanoic acid (Ahx) linkers for efficient recognition of the tag. UbiQ-187 can be used for activity profiling experiments and determining DUB inhibitor specificity.

A

YPYDVPDYA-Ahx-Ahx-
NleQIFVKTLTGKTITLEVEPSDTIENVKAKIQDKEGIPPDQQRLIFAGKQLEDGRTLSDYNIQKESTLHLVLRLRG 

B

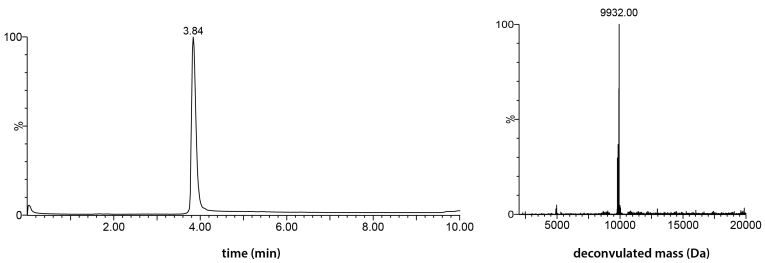


Figure 1. A: sequence; to eliminate Met1 oxidation, Met1 is replaced by norleucine, a well validated methionine mimic. B: LC-MS analysis. Mobile phase A= 1% CH_3CN, 0.1% formic acid in water and B= 1% water and 0.1% formic acid in CH_3CN. XBridge BEH300 C18 5µm 4.6x100mm; flow rate = 0.8 mL/min, runtime = 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 (e.g. 20 – 40 mg/mL)
- add this DMSO stock slowly to milliQ (please note the order of addition)
- buffer the aq. solution as desired
- for detailed experimental conditions please see open-access reference 1

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

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