UbiQ
targeting the ubiquitin system

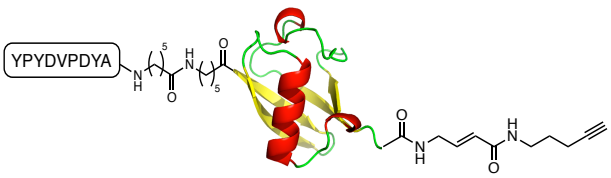

Figure 1

HA-Ahx-Ahx-Ub-VPA *(VPA= vinyl pentynyl amide, human sequence, synthetic)*

UbiQ code : UbiQ-219
Batch # : B01045014-001
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. HA-Ahx-Ahx-Ub-VPA (UbiQ-219, Figure 1) is an activity-based probe for deubiquitinating enzymes (DUBs). It is labelled 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. The alkyne group in the vinyl pentynyl amide warhead allows for further (post-labelling) modification by using click chemistry.

sequence

YPYDVPDYA-**Ahx-Ahx-**
MQIFVKTLTGKTITLEVEPSDTIENVKAKIQDKEGIPPDQQRLIFAGKQLEDGRTLSDYNIQKESTLHLVLRLRG-**VPA**

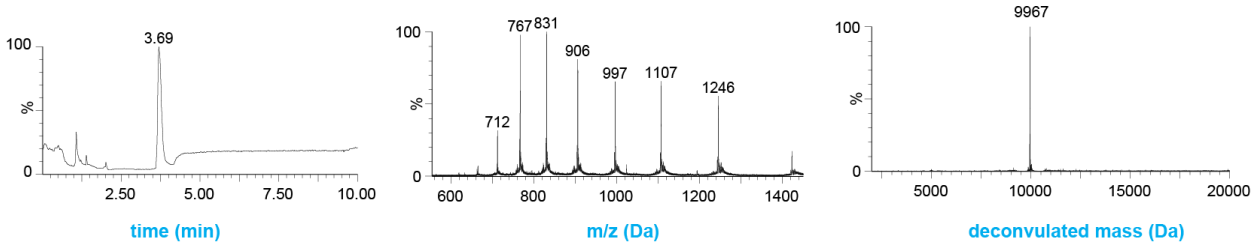

Figure 2 - LC-MS analysis. XBridge BEH300 C18 5µm 4.6x100 mm column; flow rate = 0.8 mL/min, runtime = 10 min, column T= 40°C. Mobile phase A = 1% CH_3CN and 0.1% formic acid in water; B= 1% water and 0.1% formic acid in CH_3CN. Gradient: 30-60% B over 6.5 min.

Important: sample preparation

- dissolve the powder in as little DMSO as possible (e.g., 20 mg/mL)
- 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

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.

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