UbiQ
targeting the ubiquitin system

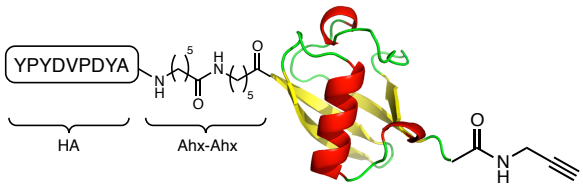

Figure 1.

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

UbiQ code	:	UbiQ-078
Batch #	:	B01122020-001
Amount	:	50 ug, lyophilized powder
Purity	:	≥95% by RP-HPLC
Mol. Weight	:	9.85 kDa
Storage	:	upon arrival, powder at −20°C; solution at −80°C. Please avoid multiple freeze/thaw cycles.

Productsheet

Background. UbiQ-078 is an activity-based probe for deubiquitinating enzymes (DUBs). It 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 aminohexanoic acid (Ahx) linkers for efficient recognition of the tag. UbiQ-078 can be used for activity profiling experiments and determining DUB inhibitor specificity. The PA group has two unique capabilities: first, it forms a covalent linkage with (the active site Cys residue of) a DUB that can be cleaved by acid treatment (5% aq. TFA), allowing for proteomic analyses; secondly, it targets all three major DUB families: UCH, USP and OUT. Although Ub-PA based probes mainly target DUBs, the active-site Cys residue of certain HECT E3 ligases (HUWE1 and NEDD4) has been found to react with Ub-PA; as such Ub-PA based probes can also be used and evaluated for the study of HECT E3 ligases. For more details, please see reference 5 (Nair et al.).

A

YPYDVPDYA-**Ahx-Ahx-**
MQIFVKTLTGKTITLEVEPSDTIENVKAKIQDKEGIPPDQQRLIFAGKQLEDGRTLSDYNIQKESTLHLVLRLRG-**PA**

B

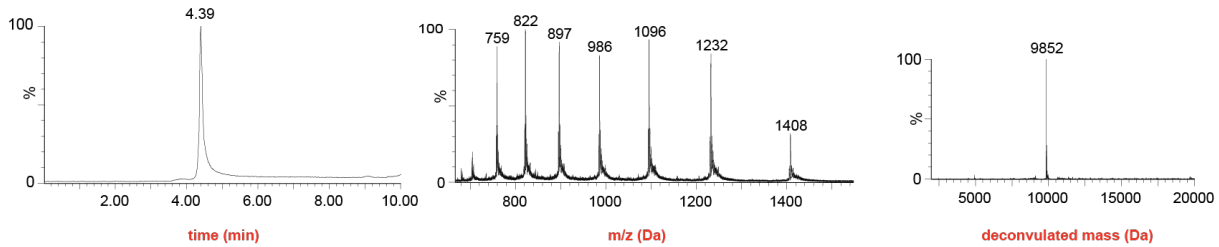

Figure 2. A: sequence UbiQ-078. B: LC-MS analysis. Mobile phase A= 1% CH_3CN, 0.1% formic acid in water (milliQ) and B = 1% water (milliQ) and 0.1% formic acid in CH_3CN. XBridge BEH300 C18 5µm 4.6x100mm; column T = 40°C, flow= 0.8 mL/min. Gradient: 20–50%B over 6.5 min.

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).
- next, buffer 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) Rodenko et al. *Nat Prot* **2006**, *1*, 1120. (2) Aoki et al *Bioorg Med Chem* **2009**, *17*, 3405. (3) Ekkebus et al. *J Am Chem Soc* **2013**, *135*, 2867. (4) Sommer et al. *Bioorg Med Chem* **2013**, *21*, 2511. (5) Nair et al. *ACS Chem Biol* **2021**, *16*, 1615.

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