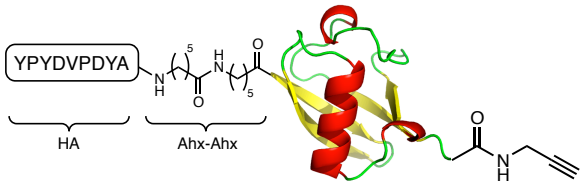
UbiQ
targeting the ubiquitin system

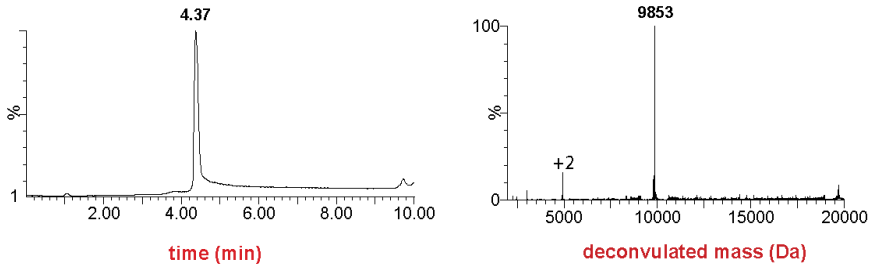


HA-Ahx-Ahx-Ub-PA *(human sequence, synthetic)*

UbiQ code	:	UbiQ-078
Batch #	:	B01052014-001
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 (Figure 1) is a potent and specific inhibitor of deubiquitinating enzymes (DUBs) containing the propargyl amide (PA) as a newly discovered DUB activity warhead.[1,2] It can be used for activity profiling experiments and determining DUB inhibitor specificity,[1-3] using two unique capabilities of the PA warhead: 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 (Fig 1A).[1] Secondly, it targets all three major DUB families: UCH, USP and OTU (Fig 1BC).[1] **UbiQ-078** is *N*-terminally tagged 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 Ub *N*-terminus by two aminohexanoic acid (Ahx) linkers for efficient recognition of the tag.



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% 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.[1-3] 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.
- if required, total removal of DMSO is accomplished by dialysis or spin-filtration (3 kDa cut-off membrane).
- for detailed experimental conditions please see open-access reference 1

* please note: Ub proteins tend to show aggregation 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 and/or add 4M urea to your SDS-PAGE analysis samples.

Literature. (1) Ekkebus et al. *J Am Chem Soc* **2013**, *135*, 2867: http://onlinelibrary.wiley.com/doi/10.1002/cbic.201200497/abstract.
(2) Sommer et al. *Bioorg Med Chem* **2013**, *21*, 2511. (3) de Jong et al. *ChemBioChem* **2012**, *13*, 2251.

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