

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

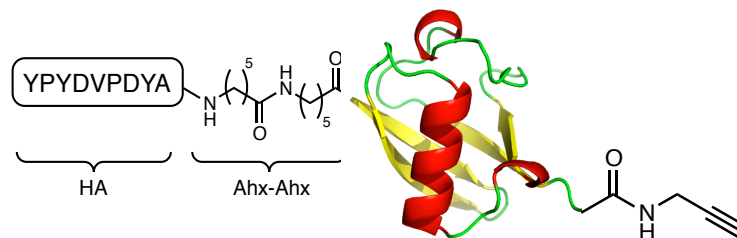
UbiQ code : UbiQ-078
Batch # : B01052014-001
Amount : 50 ug, lyophilized powder
Purity : ≥95% by RP-HPLC
Mol. Weight : 9852 Da by MS (calc Mw 9852 Da)
Storage : upon arrival powder at -20°C; solution at -80°C. Avoid multiple freeze/thaw cycles.

Productsheet

Background. UbiQ-078 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.

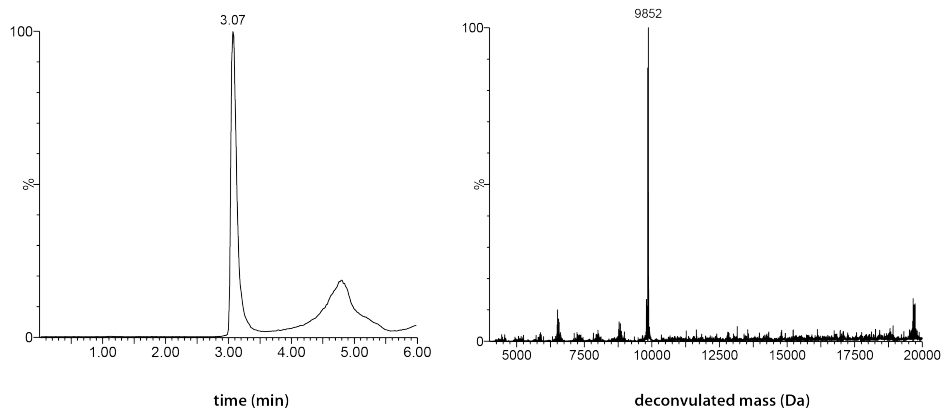
Sequence

YPYDVPDYA-(Ahx)₂-MQIFVKLTGKTTITLEVEPSDTIENVKAKIQDKEGIPPDQQRLLIFAGKQLEDGRTLSDYNIQKESTLHLV LRLRG-PA



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 with e.g. 1M HEPES to 50 mM HEPES. 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; in general DMSO concentrations of up to 5 vol% are well tolerated by DUBs.
- if required, total removal of DMSO is accomplished by dialysis or spin-filtration (3 kDa cut-off membrane).



LC-MS analysis. Mobile phase A = 1% CH₃CN, 0.1% formic acid in water (milliQ) and B = 1% water (milliQ) and 0.1% formic acid in CH₃CN. XBridge BEH300 C18 5µm 4.6x100mm; column T = 40°C, flow = 0.8 mL/min. Gradient: 30–95% over 3.5 min.

Note: higher molecular weight artefacts are observed sometimes during SDS-PAGE analysis of monoUb reagents (especially with reactive DUB activity based probes). There is no proof for these higher mol. weight bands actually being present in the material as judged by LC-MS analysis.

Literature. (1) Ekkebus et al. *J. Am. Chem. Soc.* **2013**, *135*, 2867. (2) Sommer et al. *Bioorg. Med. Chem.* **2013**, *21*, 2511. (3) de Jong et al., *ChemBioChem* **2012**, *13*, 2251.