UbiQ
targeting the ubiquitin system

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

UbiQ code	:	UbiQ-076
Batch #	:	B01082013-001
Amount	:	50 ug, lyophilized powder
Purity	:	≥95% by RP-HPLC and SDS-PAGE
Mol. Weight	:	8.89 kDa
Storage	:	upon arrival, powder at −20°C; buffered solution at −80°C. Please avoid multiple freeze/thaw cycles.

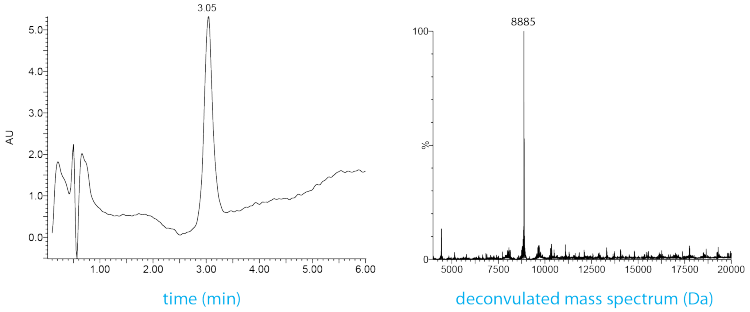
Productsheet

Background. UbiQ-076 is a potent and specific inhibitor of deubiquitinating enzymes (DUBs), which is labeled on the *N*-terminus with biotin and a *C*-terminal propargylamide (PA, alternative abbreviation= Prg). An aminohexanoic acid (Ahx) linker is used to create extra space between the biotin and Ub protein for efficient access of biotin binding entities. UbiQ-076 can be used for activity profiling experiments and determining DUB inhibitor specificity.[1-4] The PA part 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 the three major DUB families: UCH, USP and OTU.[1,2]

sequence

Biotin-Ahx-MQIFVKTLTGKTITLEVEPSDTIENVKAKIQDKEGIPPDQQRLIFAGKQLEDGRTLSDYNIQKESTLHLVLRLRG-**PA**

A



B

A: LC-MS analysis. Mobile phase A= 1% CH_3CN, 0.1% formic acid in milliQ, B= 1% milliQ and 0.1% formic acid in CH_3CN. Phenomenex Kinetex C18, (2.1×50 mm, 2.6 µM); flow rate= 0.5 mL/min, column T= 40°C. Gradient: 5% ⇨ 95% over 3.5 min. **B: SDS-PAGE analysis.** Coommassie blue staining, 12% SDS-PAGE gel.

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. (2) Sommer et al. *Bioorg Med Chem* **2013**, *21*, 2511. (3) de Jong et al. *ChemBioChem* **2012**, *13*, 2251.

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