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.

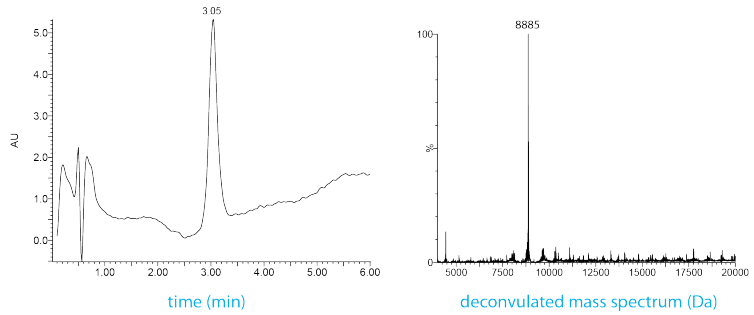
Productsheet

Background. Biotin-Ahx-Ub-PA (**UbiQ-076**) is a potent and specific inhibitor of deubiquitinating enzymes (DUBs), which is labeled on the *N*-terminus with biotin. 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] It 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 (20 - 40 mg/mL)**
- **<u>add the DMSO stock</u> to milliQ (please note the order of addition) and mix**
- **buffer the aq. solution as desired (using 1M HEPES or 1M Tris for example)**
- **in general, DMSO concentrations up to 5 vol% are well tolerated by most enzymes.**
- **If required, total removal of DMSO is accomplished by dialysis or spin-filtration (3 kDa cut-off membrane).**
- **For detailed experimental conditions please see the open-access reference 1: http://onlinelibrary.wiley.com/doi/10.1002/cbic.201200497/abstract**

Literature. (1) Ekkebus et al. *J. Am. Chem. Soc.* **2013**, *135*, 2867. (2) Sommer et al. *Bioorg. Med. Chem.* **2013**, *21*, 2511. (3) Galardy et al. *Methods in Enzymology* **2005**, *399*, 120. (4) de Jong et al. *ChemBioChem* **2012**, *13*, 2251.

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