UbiQ
targeting the ubiquitin system

**Biotin-Ahx-Ub-PA** *(human sequence, synthetic, alternative name = Biotin-Ahx-Ub-Prg)*

| | | |
|---|---|---|
| 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. |

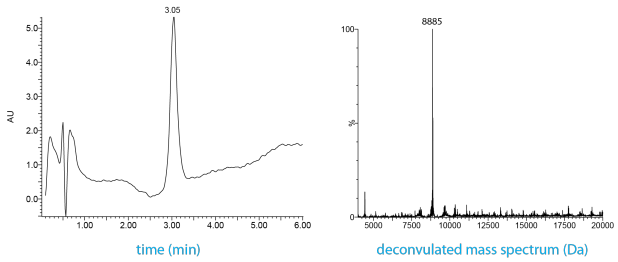
# Productsheet

**Background.** UbiQ-076 is an activity-based probe for deubiquitinating enzymes (DUBs). It is labeled on the *N*-terminus with biotin and a propargyl amide (PA) on the C-terminus. 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. 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 the PA group. As such 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.).

**sequence**

**Biotin-Ahx-**MQIFVKTLTGKTITLEVEPSDTIENVKAKIQDKEGIPPDQQRLIFAGKQLEDGRTLSDYNIQKESTLHLVLRLRG-**PA**

**A**




**B**

**Figure 1.** 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. Coomassie blue staining, 12% SDS-PAGE gel.

## 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) 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. (4) Altun et al. *Chem Biol* **2011**, *18*, 1401. (5) Nair et al. *ACS Chem Biol* **2021**, *16*, 1615.

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