UbiQ
targeting the ubiquitin system

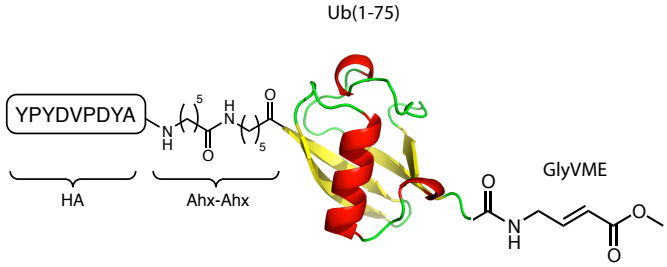


**Figure 1.**

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

| | | |
|---|---|---|
| UbiQ code | : | UbiQ-035 |
| Batch # | : | B01042014-001 |
| Amount | : | 50 ug, lyophilized powder |
| Purity | : | ≥95% by RP-HPLC |
| Mol. Weight | : | 9.91 kDa |
| Storage | : | upon arrival, powder at –20°C; solution at –80°C. Avoid multiple freeze/thaw cycles. |

# Productsheet

**Background.** UbiQ-035 is an activity-based probe for deubiquitinating enzymes (DUBs) that is based on ubiquitin functionalised with a C-terminal electrophilic vinyl methyl ester (VME) and an N-terminal HA-tag. The HA peptide sequence (YPYDVPDYA) is derived from the influenza hemagglutinin protein and allows for the sensitive identification or purification of DUBs since it is specifically recognized by anti-HA antibodies and 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.

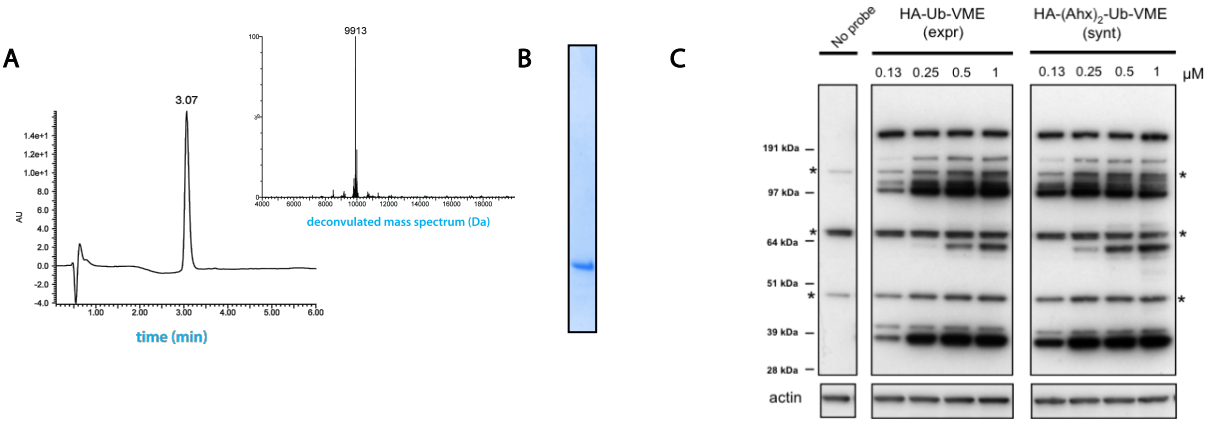


**Figure 2.** A: LC-MS analysis. Mobile phase A= 1% $CH_3CN$, 0.1% formic acid in water (milliQ) and B= 1% water (milliQ) and 0.1% formic acid in $CH_3CN$. Phenomenex Kinetex C18, (2.1×50 mm, 2.6 μM); flow rate= 0.8 mL/min, column T= 40°C. Gradient: 5-95%B over 3.5 min. B: SDS-PAGE analysis. 12% Bis-Tris, MES buffer, CBB staining. C: Comparison DUB labeling efficiency between conventional HA-Ub-VME (obtained from bacterial expressed Ub precursor)[2] and synthetic HA-Ahx-Ahx-Ub-VME (UbiQ-035). EL4 cell lysate was incubated at ambient temperature for 15 min. with indicated concentrations of probe; both probes (i.e., expressed and synthetic UbiQ-035) showed comparable DUB labeling.
*= background bands due to cross-reactivity of anti-HA antibody.

## 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

Note: higher molecular weight artefacts are observed sometimes during SDS-PAGE analysis of monoUb reagents (especially with reactive DUB activity-based probes). 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 loading buffer.

**Literature.** (1) de Jong et al. *ChemBioChem* **2012**, *13*, 2251. (2) El Oualid et al. *Angew Chem Int Ed* **2010**, *49*, 10149.

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