UbiQ
targeting the ubiquitin system

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

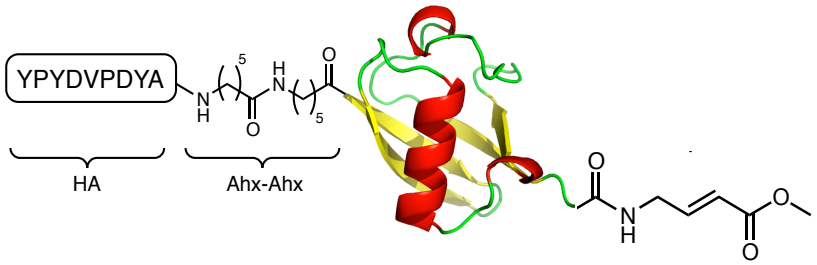
UbiQ code	:	UbiQ-035
Batch #	:	B01042014-001
Amount	:	50 ug, lyophilized powder
Purity	:	≥95% by RP-HPLC
Mol. Weight	:	9913 Da by MS (calc Mw 9912 Da)
Storage	:	upon arrival powder at −20°C; solution at −80°C. Avoid multiple freeze/thaw cycles.

Productsheet

Background. A potent, irreversible and specific inhibitor of deubiquitinating enzymes (DUBs)[1] that is prepared by chemical synthesis.[2] It is *N*-terminally tagged with an 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.

Sequence

YPYDVPDYA-(Ahx)$_2$-MQIFVKTLTGKTITLEVEPSDTIENVKAKIQDKEGIPPDQQRLIFAGKQLEDGRTLSDYNIQKESTLHLV LRLRG-VME



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).**

UbiQ Bio BV
Chamber of Commerce 50023438
VAT NL822502136B01

Science Park 408
1098 XH Amsterdam
The Netherlands

t +31 20 303 1970
e info@ubiqbio.com
i www.ubiqbio.com

Rabobank
IBAN NL86RABO0150658907
BIC/SWIFT RABONL2U

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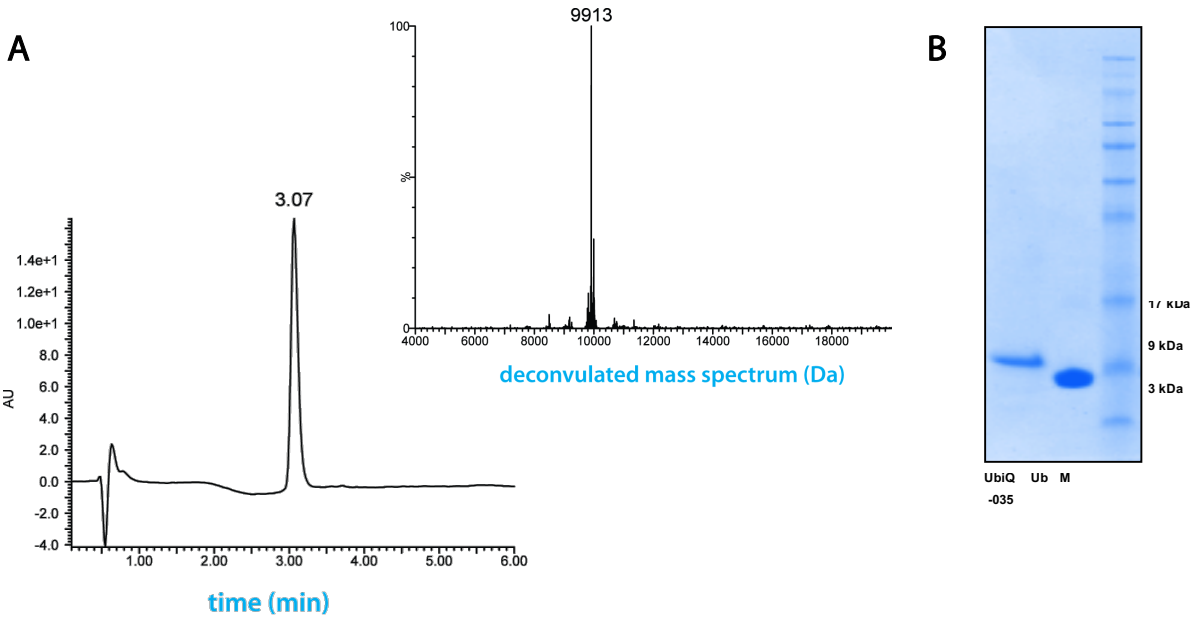

A: LC-MS analysis. Mobile phase A = 1% CH3CN, 0.1% formic acid in water (milliQ) and B = 1% water (milliQ) and 0.1% formic acid in CH_3CN. Phenomenex Kinetex C18, (2.1×50 mm), 2.6 µM); flow rate= 0.8 mL/min, runtime= 6 min, column T= 40°C. Gradient: 0 – 0.5 min: 5% B; 0.5 – 4 min: 5% ⇨ 95% B; 4 – 5.5 min: 95% **B: SDS-PAGE analysis.** 12% Bis-Tris, MES buffer **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.

Literature. (1) (a) de Jong et al. *ChemBioChem* **2012**, *13*, 2251. (b) Borodovsky et al. *EMBO J.* **2001**, *20*, 5187. (c) Borodovsky et al. *Chemistry and Biology* **2002**, *9*, 1149. (2) El Oualid et al. *Angew. Chem. Int. Ed.* **2010**, *49*, 10149.

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