UbiQ
targeting the ubiquitin system

K63 di-Ubiquitin VME *(human sequence, synthetic)*

UbiQ code	:	UbiQ-087
Batch #	:	B15102014-001
Amount	:	50 ug, lyophilized powder
Purity	:	≥90% by RP-HPLC and SDS-PAGE analysis
Mol. Weight	:	17.11 kDa
Storage	:	upon arrival, powder at −20°C and solutions at −80°C. Please avoid multiple freeze/thaw cycles.

Productsheet

Background. UbiQ-087 is an activity-based probe for deubiquitinating enzymes (DUBs) based on K63 linked diUb. Here Lys63 has been replaced by a diaminobutyric acid residue equipped with a vinyl methyl amide warhead - the Dab(VME) type of structure is a DUB reactive mimic of the native isopeptidic linked Lys(Gly) residue (Figure 1B). DUB activity based probe can be used for DUB activity profiling experiments (Figure 2) and structural studies. Please note the native distance between the proximal and distal Ub is preserved as much as possible in UbiQ-087 (Figure 1B).

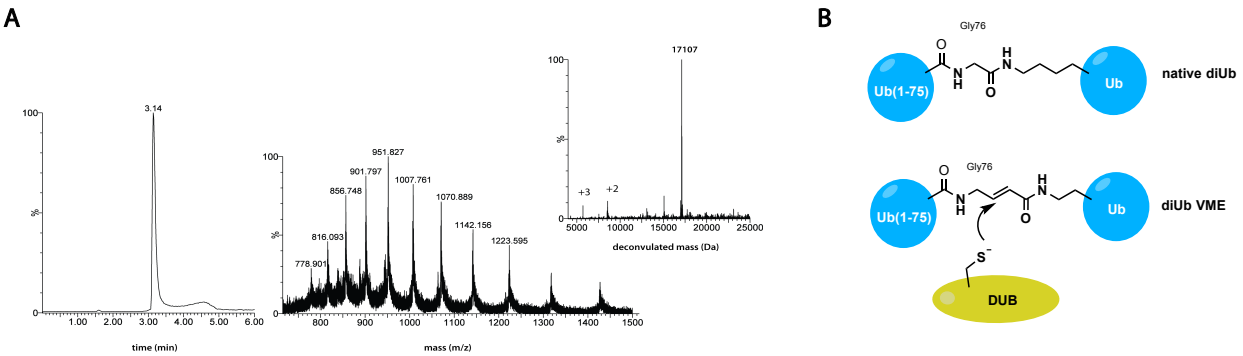

Figure 1: A: LC-MS analysis. Mobile phase A= 1% CH_3CN, 0.1% formic acid in milliQ; B= 1% milliQ, 0.1% formic acid in CH_3CN. XBridge BEH300 C18 5µm 4.6x100mm; column T= 40°C, flow= 0.8 mL/min. Gradient: 30–95% over 3.5 min. **B=** mode of action diUb VME labeling of DUBs.

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) and mix by vortexing
- buffer the aq. stock as desired (with e.g. 1M HEPES or Tris, pH 7.5 - 8)
- For more details see (open-access) reference 1: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4159580/

UbiQ Bio BV
Chamber of Commerce 50023438
VAT NL822502136B01

Science Park 301
1098 XH Amsterdam
The Netherlands

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

Rabobank
IBAN: NL86 RABO 0150658907
BIC/SWIFT: RABONL2U

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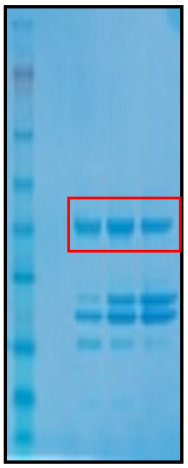

1= UbiQ-087 B15102014-001/USP4 **ratio ±5/4**
2= UbiQ-087 B15102014-001/USP4 **ratio ±5/8**
3= UbiQ-087 B15102014-001/USP4 **ratio ±2.5/8**

Figure 2: Labeling experiment with USP4 (D1D2 dimer). Reaction conditions: 100 µg UbiQ-087 was dissolved in 4 uL DMSO and added to 96 uL milliQ. This aq. stock was buffered to 100 mM HEPES, 100 mM NaCl pH 8 to a final cocn of 0.5 mg/mL=29 uM. Next, 1 uL of a 1M TCEP stock pH 7 was added for a final TCEP conc. of 5 mM. Three DUB raction mixtures were prepared with increasing DUB ratio.

- 10 uL UbiQ-087 B15102014-001 (5 ug, ±0.36 mg/mL) + 4 uL USP4 stock (4 ug, 0.29 mg/mL)= lane 10 gel
- 10 uL UbiQ-087 B15102014-001 (5 ug, ±0.28 mg/mL) + 8 uL USP4 stock (8 ug, 0.44 mg/mL)= lane 11 gel
- 5 uL UbiQ-087 B15102014-001 (2.5 ug, ±0.19 mg/mL) + 8 uL USP4 stock (8 ug, 0.62 mg/mL)= lane 12 gel

The reactions were incubated at 37°C for 2 hrs, quenched by the addition of reducing sample buffer and heated at 90°C for 10 min. Samples were analyzed by SDS-PAGE analysis using a 12% Bolt Bis-Tris Plus gel (Life technologies) and MES running buffer. M= SeeBlue Plus2 Pre-stained Standard marker (Invitrogen). CBB staining was performed with a *Coommassie G-250* solution (80 mg in 1L water + 3 mL HCl). Note that according to SDS-PAGE analysis the material contains some Ub(1-75). This originates from the initial synthesis step of the probe and contains no DUB reactive groups - thus only labeling with the diUb probe is observed.

Literature. (1) Mulder & El Oualid et al. *ChemBioChem* **2014**, *15*, 946.

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