UbiQ
targeting the ubiquitin system

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

UbiQ code	:	UbiQ-087
Batch #	:	B15102014-001
Amount	:	25 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 a potent, irreversible and specific inhibitor of deubiquitinating enzymes (DUBs) based on K63 linked diUb.[1] Here Lys63 has been replaced by a diaminobutyric acid residue equipped with a VME type warhead[3] - the Dab(VME) type of structure is a DUB reactive mimic of the native isopeptidic linked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.[1-8] Please note the native distance between the proximal and distal Ub is preserved as much as possible in UbiQ-087 (Figure 1B).

Sequence

MQIFVKTLTGKTITLEVEPSDTIENVKAKIQDKEGIPPDQQRLIFAGKQLEDGRTLSDYNIQKESTLHLVLRLRG-

MQIFVKTLTGKTITLEVEPSDTIENVKAKIQDKEGIPPDQQRLIFAGKQLEDGRTLSDYNIQ(**Dab(VME)**)ESTLHLVLRLRGG

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**
- **final stocks of e.g. 0.5 mg/mL will contain 2.5 vol% DMSO.**
- **buffer the aq. stock as desired (with e.g. 1M HEPES or Tris, pH 7.5 - 8)**
- **please note optimal buffer conditions can vary between DUBs - if this is unknown for your DUB, we advise to determine this first (using e.g. other simple DUB probes like Ub-VME)**
- **in general, a DMSO concentration up to 5 vol% is well tolerated by DUBs**
- **For more details see (open-access) reference: http://www.ncbi.nlm.nih.gov/pubmed/24623714**

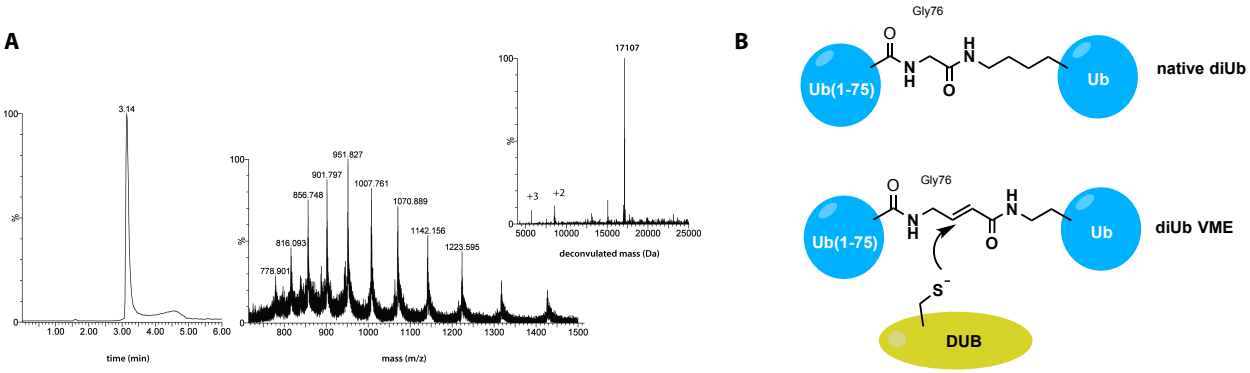

Figure 1: A=LC-MS analysis. Mobile phase A= 1% CH_3CN, 0.1% formic acid in milliQ and B= 1% milliQ and 0.1% formic acid in CH_3CN. XBridge BEH300 C18 5µm 4.6x100mm; column T= 40°C, flow= 0.8 mL/min. Gradient: 30–95% over 3.5 min.
B= mode of action diUb VME labeling of DUBs.

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UbiQ
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1= 4 ug UbiQ-087 B15102014-001
2= 5 ug UbiQ-087 B15102014-001
3= 1 ug K63 diUb (Boston Biochem)
4= 2 ug K63 diUb (Boston Biochem)
5= 3 ug K63 diUb (Boston Biochem)
6= 4 ug K63 diUb (Boston Biochem)

7= UbiQ-087 B15102014-001/USP4 **ratio ±5/4**
8= UbiQ-087 B15102014-001/USP4 **ratio ±5/8**
9= UbiQ-087 B15102014-001/USP4 **ratio ±2.5/8**

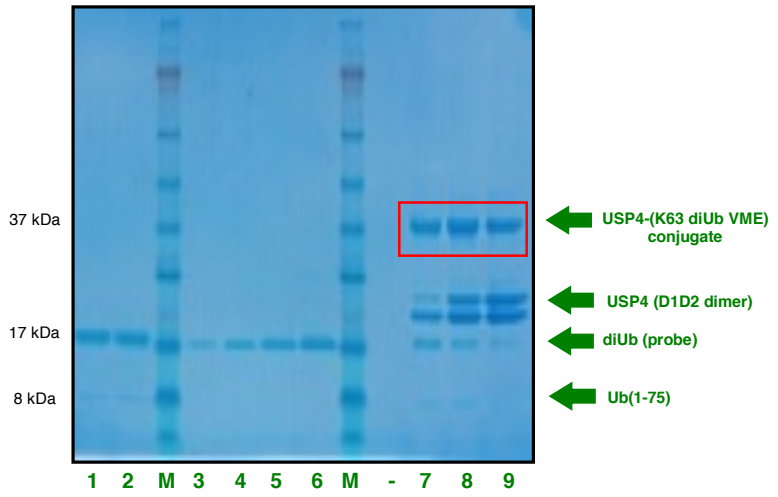

Figure 2: Labeling experiment with USP4 (D1D2 dimer). Reaction conditions: 100 µg UbiQ-087 was dissolved in 4 uL DMSO and added to 96 uL milliQ. This aq. stock was buffered to 100 mM HEPES, 100 mM NaCl pH 8 to a final cocn of 0.5 mg/mL=29 uM. Next, 1 uL of a 1M TCEP stock pH 7 was added for a final TCEP 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. Marker= SeeBlue Plus2 Pre-stained Standard (Invitrogen). CBB staining was performed with a *Coommassie G-250* solution (80 mg in 1L water + 3 mL HCl). Protein content of **UbiQ-087** was verified with commercial K63 diUb (Boston Biochem). *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. (2) Misaghi et al. *J. Biol. Chem.* **2005**, *280*, 1512. (3) de Jong et al. *ChemBioChem* **2012**, *13*, 2251. (4) Altun et al. *Chem. Biol.* **2011**, *18*, 1401. (5) Haj-Yahya et al. *Org. Lett.*, **2014**, *16*, 540. (6) Li et al. *Chem. Commun.* **2014**, *50*, 216. (7) Iphöfer et al. *ChemBioChem* **2012**, *13*, 1416. (8) McGouran et al. *Chem. Biol.* **2013**, *20*, 1447.

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