UbiQ
targeting the ubiquitin system

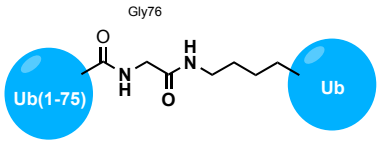
Biotin-Ahx-K63 di-Ubiquitin VME *(human sequence, synthetic)*

UbiQ code	:	UbiQ-115
Batch #	:	B01062015-001
Amount	:	50 ug, lyophilized powder
Purity	:	≥95% by SDS-PAGE analysis
Mol. Weight	:	17.45 kDa
Storage	:	upon arrival, powder at −20°C, solution at −80°C. Please avoid multiple freeze/thaw cycles.

Productsheet

Background. UbiQ-115 is a potent and specific inhibitor of deubiquitylating enzymes (DUBs) based on K63 linked diUb. UbiQ-115 contains a Dab(VME) electrophile that labels Cys based DUBs in an irreversible covalent manner (Figure 1). In the case of DUBs with no nucleophilic catalytic residue (such as the metallo-DUBs), UbiQ-115 can be used as a reversible binding inhibitor. DUB activity-based probes such as UbiQ-115 can be used for activity profiling experiments, as pull-down tools for K63 diUb binding proteins and structural studies. In UbiQ-115 the Lys63 residue is replaced by a diaminobutyric acid residue equipped with a VME type electrophile - this Dab(VME) structure (Figure 1) is a DUB resistant mimic of the native isopeptidic linked Lys(Gly) residue. The native distance between the proximal and distal Ub is preserved as much as possible and the N-terminus of the distal Ub is labeled with biotin; an aminohexanoic acid (Ahx) linker creates extra space for efficient access of biotin binding entities. UbiQ-115 has been prepared by total chemical synthesis and is therefore well-defined in terms of biotinylation site. Its reactivity and resistance to DUBs has been validated with USP4 (Figure 2).

A



B

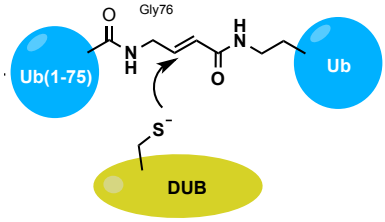


Figure 1. A: native diubiquitin. B: diUb VME probe

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)
- 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

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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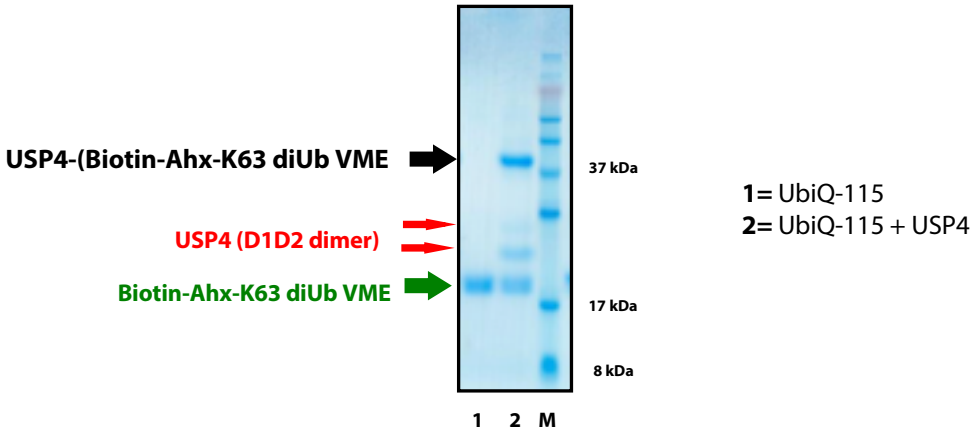

Figure 2. Labeling experiment of UbiQ-115 with USP4 (D1D2 dimer).

Experimental conditions:

- 100 µg of UbiQ-115 was dissolved in 5 uL DMSO, added to 90 uL milliQ and dissolved by vortexing.
- 5 uL of a 1M HEPES pH stock is added followed by 2 uL of a 5M NaCl stock and 1 uL of a 1M TCEP pH 7 stock.
- this results in a 1 mg/mL stock of UbiQ-115 (57 uM) in 50 mM HEPES, 100 mM NaCl, 10 mM TCEP pH 8.
- next, 4 uL of this stock (= 4 ug UbiQ-115) was added to 5 ul 50 mM HEPES, 100 mM NaCl pH 8 and treated with 1 ug USP4 (1 uL of 1 mg/mL stock).
- this results in a 0.5 mg/mL stock of UbiQ-115 (29 uM) in 50 mM HEPES, 100 mM NaCl, 5 mM TCEP pH 8.
- the reaction was incubated at 37°C for 2 hrs, quenched with 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 Coommassie G-250.

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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The Netherlands

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

Rabobank
IBAN NL86RABO0150658907
BIC/SWIFT RABONL2U