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 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 Here Lys63 has been replaced by a diaminobutyric acid residue equipped with a vinyl methyl amide warhead - the Dab(VME) type of structure is a DUB reactive mimic of the native isopeptidic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. The native distance between the proximal and distal Ub is preserved as much as possible in UbiQ-115 (Figure 1B). 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.

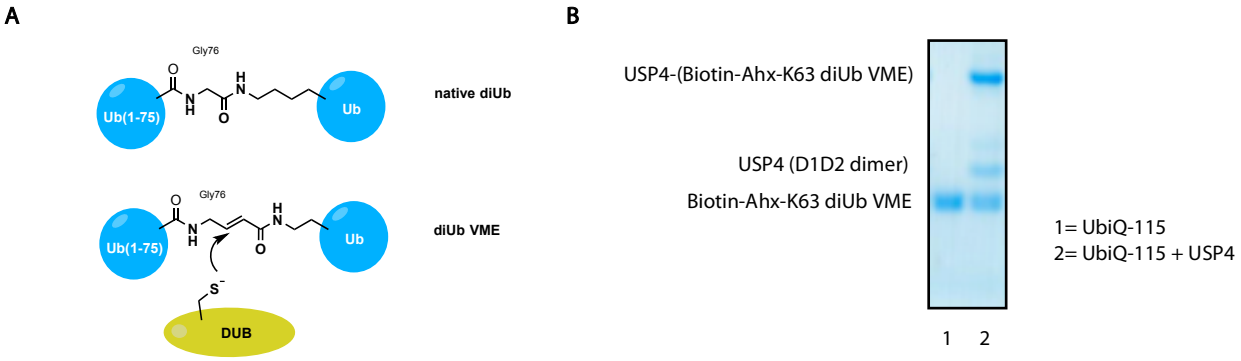

Figure 1. A: Mode of action diUb VME labeling of DUBs. Top= native diUb, bottom= diUb VME probe. B: Labeling experiment of UbiQ-115 with USP4 (D1D2 dimer). Experimental conditions: 100 µg of UbiQ-115 was dissolved in 5 uL DMSO, added to 90 uL milliQ and dissolved by vortexing; 5 uL of a 1M HEPES pH stock 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). Coommassie G-250 staining.

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
- For more details see (open-access) reference 1: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4159580/

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

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