UbiQ
targeting the ubiquitin system

Di-Ubiquitin VME explorer panel *(human sequence, synthetic)*

UbiQ code	:	UbiQ-L04
Batch #	:	B01022015-001
Amount	:	7×10 µg lyophilized powder: K6, K11, K27, K29, K33, K48 and K63 linked diUb VME
Purity	:	±90% by RP-HPLC and SDS-PAGE analysis*
Mol. Weight	:	17.11 kDa
Storage	:	upon arrival powder at −20°C; solution at −80°C. Please avoid multiple freeze/thaw cycles.

Productsheet

Background. UbiQ-L04 contains a panel of seven potent, irreversible and specific inhibitors of deubiquitinating enzymes (DUBs) based on the diUb structure.[1] Here, a Lys residue has been replaced by a diaminobutyric acid residue equipped with a VME type warhead (Figure 1A).The Dab(VME) electrophile used to trap the active site cysteine of the DUB of structure is a DUB reactive mimic of the native isopeptidic linked Lys(Gly) residue (Figure 1A). The DUB activity based probes can be used for activity profiling experiments and structural studies.[1-8] Please note the native distance between the proximal and distal Ub is preserved as much as possible in our diUb VME probes (Figure 1A).

For experimental details please see (open-access) reference 1.
Pubmed link: http://www.ncbi.nlm.nih.gov/pmc/articles/PMC4159580/

Important: sample preparation

- **add 0.5 µL DMSO to the 10 µg diUb VME pellet and dissolve by a quick spin in the (ultra)centrifuge.**
- **add the DMSO stock (= 1169 µM) to the required buffer (please note order of addition) - *for convenience one can use the lid of the eppendorf tube to hold the buffer while adding the 0.5 µL DMSO.***
- **as an example, dilution of the 0.5 µL DMSO stock into 20 µL buffer affords a diUb solution of 30 µM with 2.5 vol% DMSO.**
- **Buffer exchange using 3 kDa spin filters (or dialysis membrane) can be used to remove the DMSO if desired.**

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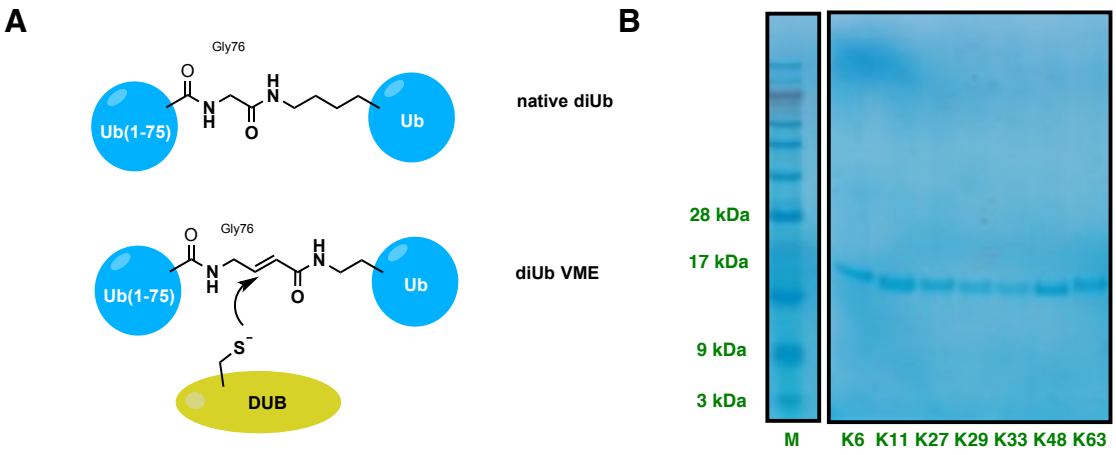

Figure 1. A: Design and mode of action diUb VME probes. **B: SDS-PAGE analysis treatment of UbiQ-L04 diUb explorer panel.**** Samples were heated at 90°C for 10 min and run on a 12% Bolt Bis-Tris Plus gel (Lifetechnologies) in MES buffer at 190V. Staining was performed with Coomassie Brilliant Blue G-250. Marker= SeeBlue® Plus2 (Invitrogen).

* Based on SDS-PAGE analysis there is some Ub(1-75) present in the sample but this does not interfere with labeling experiments with DUBs.

** In some cases we and others have observed the appearance of higher mol. weight bands ("smearing") during SDS-PAGE analysis of (di)Ub conjugates. We do not have (analytical) evidence these are actual contaminants present in the diUb sample but that they are aggregates formed during SDS-PAGE. We have also not witnessed any effect of this phenomenon on experiments performed with our diUb material.

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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