UbiQ
targeting the ubiquitin system

K63 di-Ubiquitin PA *(human, synthetic)*
UbiQ code : UbiQ-114
Batch # : B01062015-001
Amount : 25 ug, lyophilized powder
Purity : ≥95% by LC-MS and SDS-PAGE analysis
Mol. Weight : 17.1 kDa (calc 17.1 kDa)
Storage : upon arrival store solution at –80°C.

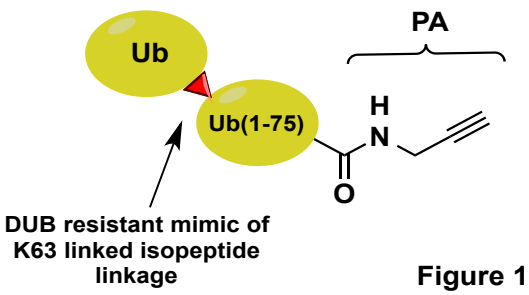
Productsheet

Background. UbiQ-114 (Figure 1A) is a newly developed potent, irreversible and specific inhibitor of deubiquitinating enzymes (DUBs) based on K63 linked diUb. The C-terminus of the proximal Ub is equiped with the novel propargylamide (PA) electrophile[1] and the isopeptide bond between the two Ub proteins is replaced by a DUB resistant mimic. This DUB activity based probe can be used for activity profiling experiments and structural studies. Please note the native distance between the proximal and distal Ub is preserved as much as possible. UbiQ-114 has been prepared by total chemical synthesis.

Important: sample preparation.

- dissolve the powder in as little DMSO as possible (e.g. 20 mg/mL) and add this DMSO stock slowly to milliQ (please note the order of addition).
- buffer with e.g. 1M HEPES to 50 mM HEPES. In general HEPES and Tris buffers are standard for DUB assays.
- add NaCl if required (e.g. from a 5M stock). Please note that certain DUBs react different to low or high NaCl concentrations.
- 2 - 5 mM DTT can be used as reducing agent for the DUB.
- a final buffered stock of for example 0.5 mg/mL contains 2.5 vol% DMSO; in general DMSO concentrations of up to 5 vol% are well tolerated by DUBs.
- if required, total removal of DMSO is accomplished by dialysis or spin-filtration (3 kDa cut-off membrane).

A



B

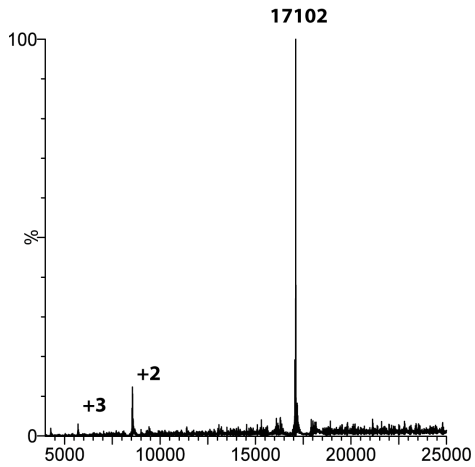


Figure 1

Figure 1. A: Design UbiQ-114. **B:** MS analysis UbiQ-114.

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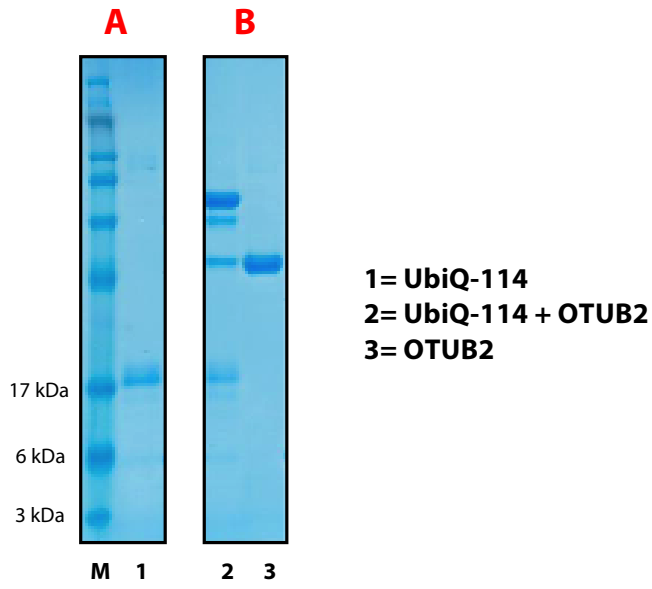

Figure 2. A: SDS-PAGE analysis of UbiQ-114; 12% Bolt Bis-Tris Plus gel (Life technologies) and MES running buffer. Marker= SeeBlue Plus2 Pre-stained Standard (Invitrogen). **B:** Labeling of OTUB2 with UbiQ-114: a 1 mg/mL stock of UbiQ-114 in 100 mM HEPES, 100 mM NaCl was prepared as outlined on page 1. Next, 4 uL of this stock (= 4 ug UbiQ-114) was added to 13 uL 100 mM HEPES pH 8, 100 mM NaCl, 5 mM DTT. To this solution was added 4 ug OTUB2 (3 uL of 1.4 mg/mL stock) and the reaction was incubated at 37°C. After 1 hr the reaction was quenched by the addition of reducing sample buffer and heated at 90ºC 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.

Literature. (1) Ekkebus et al. *J. Am. Chem. Soc.* **2013**, *135*, 2867. (2) Sommer et al. *Bioorg. Med. Chem.* **2013**, *21*, 2511. (3) Galardy et al. *Methods in Enzymology* **2005**, *399*, 120. (4) de Jong et al. *ChemBioChem* **2012**, *13*, 2251.

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