UbiQ
targeting the ubiquitin system

Di-Ubiquitin Explorer panel *(human sequence)*

UbiQ code	:	UbiQ-L01
Batch #	:	B01052014-001
Amount	:	8 x 10 µg lyophilized powder, M1 (i.e. linear), K6, K11, K27, K29, K33, K48 and K63 linked diUb
Purity	:	≥95% by RP-HPLC and LC-MS analysis, ≥95% by SDS-PAGE*
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-L01 is a panel of all eight native linked di-ubiquitin (diUb) conjugates: M1 (i.e. linear), K6, K11, K27, K29, K33, K48 and K63 linked. The seven isopeptide linked diUb conjugates have been prepared by chemical ligation, the linear (M1) diUb conjugate by recombinant expression. UbiQ-L01 can be used to:

- investigate linkage specificity of proteases that cleave the (iso)peptide linkage between two ubiquitin proteins.
- investigate mechanism of binding and recognition of proteins that contain ubiquitin-associated domains or ubiquitin-interacting motifs (UIMs).

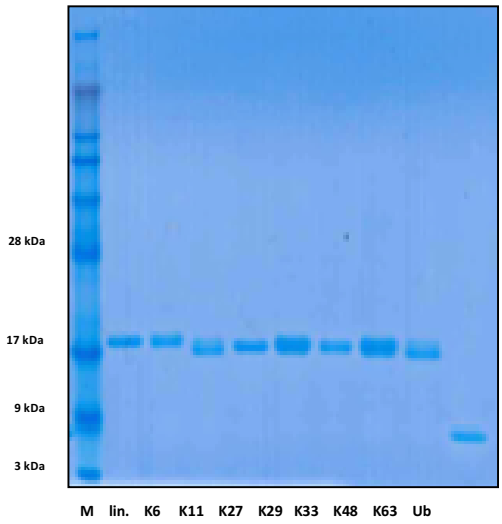
Important - Sample preparation.

- **add 0.5 µL DMSO to the 10 µg diUb sample 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.**
- **dilution of 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 can be used to remove the DMSO if desired. Please note that a DMSO conc of <5 vol% is well tolerated in most experiments (e.g. with DUBs)**

SDS-PAGE analysis*

- **1.5 ug diUb in 1x Laemmli sample buffer with 2-mercaptoethanol (15 uL) was heated at 95°C for 10 min**
- **samples were 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).**

* 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 evidence these are actual contaminants present in the (di)Ub sample. The smearing is believed to be caused by Ub aggregation during SDS-PAGE analysis. We found that heating the SDS-PAGE samples for 10 min at 95°C with Laemmli sample buffer containing reducing agent (DTT or 2-mercaptoethanol) eliminates the smearing significantly.



Literature. (1) El Oualid et al. *Angew. Chem. Int. Ed.* **2010**, 49, 10149. (2) Faesen et al. *Chemistry & Biology* **2011,**18, 1550. (3) Dikic et al. *Nature Rev. Mol. Cell Biol.* **2010**, 10, 659. (4) Licchesi et al. *Nature Struct. & Mol. Biol.* **2012**, 19, 62.

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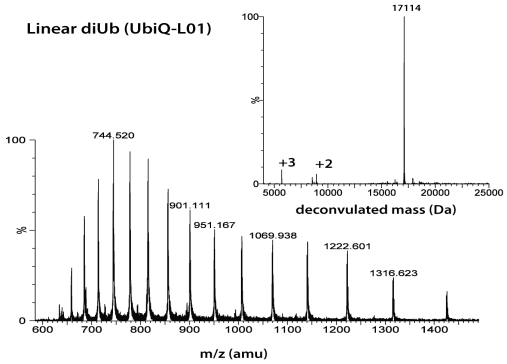

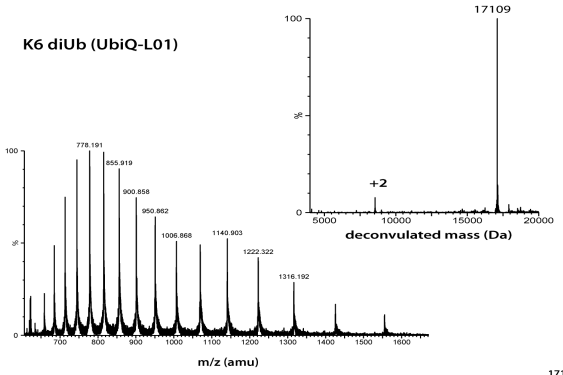

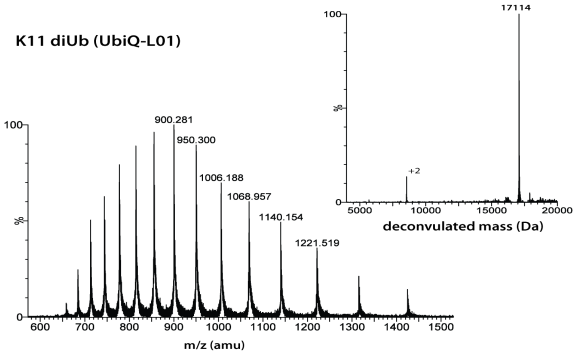

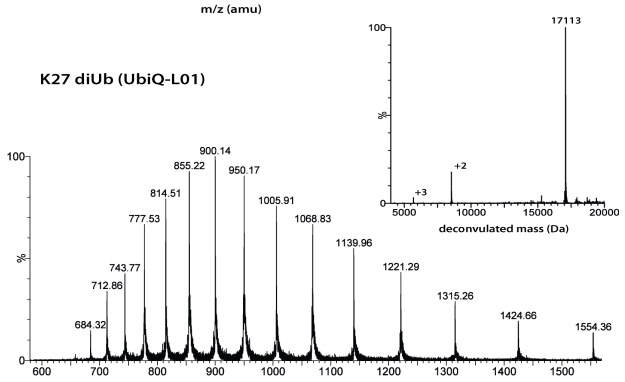

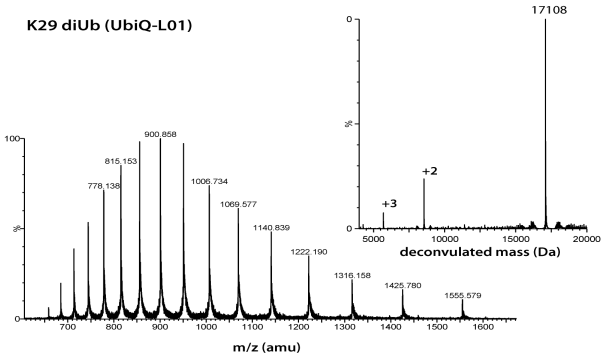

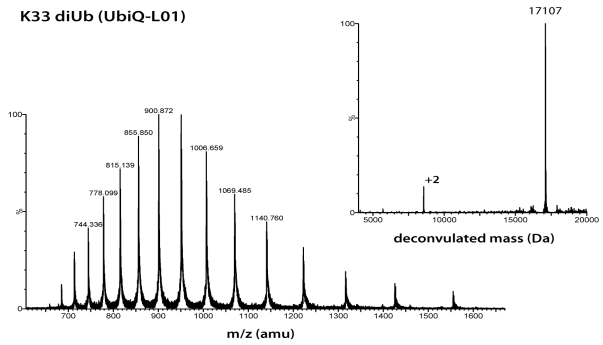

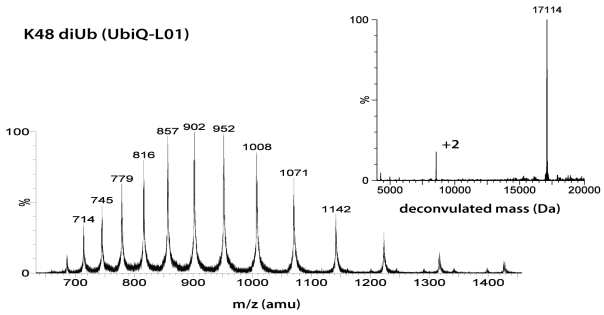

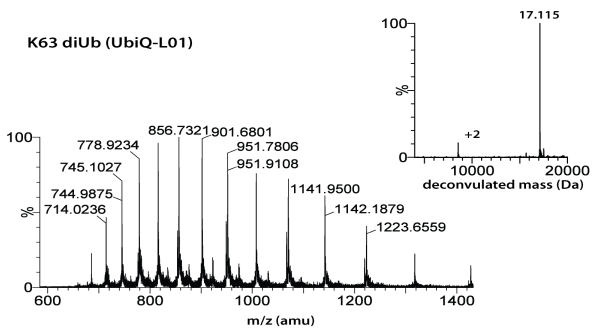

MS analysis diUb portions UbiQ-L01. Mobile phase A= 1% CH_3CN, 0.1% formic acid in milliQ and B= 1% milliQ and 0.1% formic acid in CH_3CN. Phenomenex Kinetex C18, (2.1×50 mm, 2.6 µM); flow rate = 0.5 mL/min, column T = 40°C. Gradient: 5% ⇨ 95% over 3.5 min.

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