UbiQ
targeting the ubiquitin system

Linear Di- Ubiquitin *(human sequence, recombinant)*

UbiQ code : UbiQ-070
Batch # : B01012013-001
Amount : 50 ug, lyophilized powder
Purity : ≥95% by RP-HPLC and SDS-PAGE
Mol. Weight : 17.11 kDa
Storage : upon arrival, store powder at −20°C, solution at −80°C. Please avoid multiple freeze/thaw cycles.

Productsheet

Background. UbiQ-070 is native linear (i.e. M1) linked di-Ub which can be used as a substrate for proteases that cleave the peptide linkage between two ubiquitin proteins or to investigate mechanism of binding and recognition by proteins that contain ubiquitin-associated domains or ubiquitin-interacting motifs (UIMs). This product is formed by recombinant expression (*E. coli*).

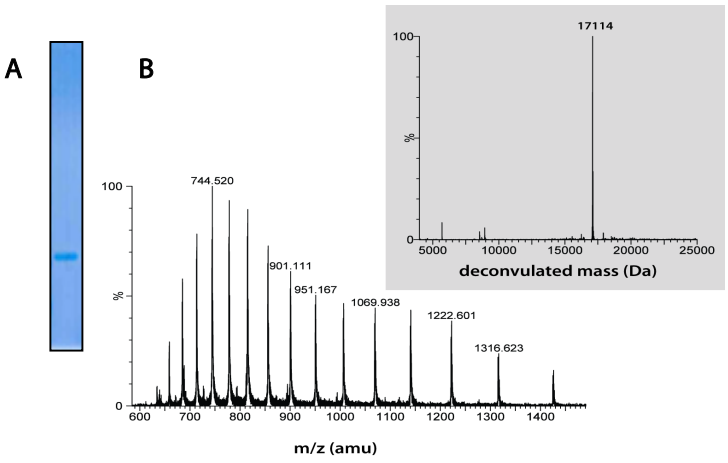
Sequence

MQIFVKTLTGKTITLEVEPSDTIENVKAKIQDKEGIPPDQQRLIFAGKQLEDGRTLSDYNIQKESTLHLVLRLRGG
MQIFVKTLTGKTITLEVEPSDTIENVKAKIQDKEGIPPDQQRLIFAGKQLEDGRTLSDYNIQKESTLHLVLRLRGG

Important - sample preparation.

- **add 2.5 µL DMSO to 50 µg diUb sample and dissolve by a quick spin in the (ultra)centrifuge.**
- **add the DMSO stock (= 1169 µM) to milliQ (please note the order of addition)**
- **buffer the aq. solution as desired**
- **dilution of 2.5 µL DMSO stock into 97.5 µL buffer affords a stock solution of 30 µM (2.5 vol% DMSO).**
- **a DMSO conc of <5 vol% is well tolerated in most experiments (e.g. with DUBs)**

A - SDS-PAGE: 12% gel, MES buffer, Coomassie Brilliant Blue staining. M= 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.*
B - MS analysis. Mobile phase A = 1% CH_3CN, 0.1% formic acid in water (milliQ) and B = 1% water (milliQ) and 0.1% formic acid in CH_3CN. Phenomenex Kinetex C18, (2.1×50 mm, 2.6 µM); flow rate = 0.6 mL/min, runtime = 6 min, column T = 40°C. Gradient: 5% ⇨ 95% B over 3.5 min.



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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