UbiQ
targeting the ubiquitin system

His6-Ahx-Ahx-SUMO2-VPS *(human sequence, C48S, synthetic)*

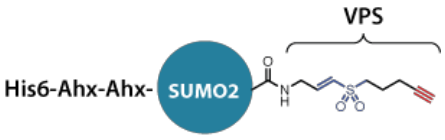


Figure 1

UbiQ code : UbiQ-237
Batch : B01112017-001
Amount : 50 ug, lyophilized powder
Purity : ≥95% by RP-HPLC
Mol. Weight : 11.8 kDa
Storage : upon arrival powder at –20°C; solution at –80°C. Avoid multiple freeze/thaw cycles.

Productsheet

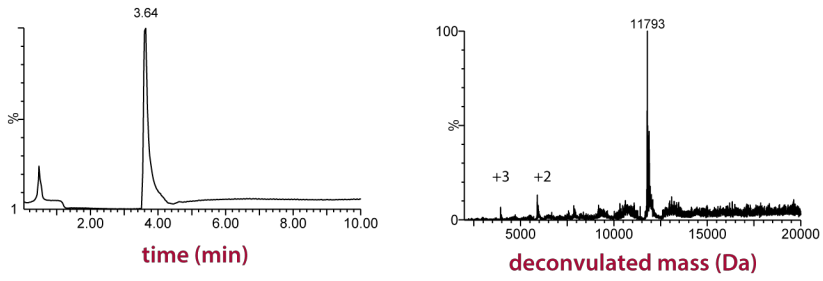
Background. UbiQ-237 is an irreversible inhibitor of SUMO proteases (SENPs) that is prepared by total chemical synthesis.[1] It contains a C-terminal vinyl pentynyl sulfone (VPS) electrophile (Figure 1) and an *N*-terminal His6 sequence* which allows for sensitive identification or purification by anti-His6 antibodies and/or anti-His6-agarose. The VPS electrophile allows for post-labeling modification of cross-linked UbiQ-237::SENP complexes by using click chemistry with for example biotin-azide.[2]

*the His6 tag is separated from the *N*-terminus by two aminohexanoic acid (Ahx) linkers for efficient recognition of the tag.

Sequence

HHHHHH-Ahx-Ahx-MADEKPKEGV KTENNDHINL KVAGQDGSVV QFKIKRHTPL SKLMKAY**S**ER QGLSMRQIRF RFDGQPINET DTPAQLEMED EDTIDVFQQQ TGG-**VPS**

A



B

A: LC-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. XBridge BEH300 C18 5µm 4.6x100mm; flow rate = 0.8 mL/min, runtime = 10 min, column T = 40°C. Gradient: 50% ⇨ 90% B over 6.5 min. **B: SDS-PAGE analysis**. 12% Bolt Bis-Tris gel (LifeTechnologies), 190 V, MES buffer. Staining with InstantBlue Protein Stain (Expedeon).

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).
- next buffer as desired. In general HEPES and Tris buffers are standard for Ubl protease assays. Please note that certain DUBs react different to low or high NaCl concentrations.
- a final buffered stock of for example 0.5 mg/mL contains 2.5 vol% DMSO.
- total removal of DMSO can be accomplished by dialysis or spin-filtration (3.5 kDa cut-off membrane).
- please be aware of background bands due to cross-reactivity of anti-HA antibodies.
- For full experimental details please see reference 2.

Literature. (1) El Oualid et al. *Angew. Chem. Int. Ed.* **2010**, *49*, 10149. (2) Hewing et al. *Nat. Comm.* **2018,** *9*, article number: 1162.

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