UbiQ
targeting the ubiquitin system

Cy5-Ub-VME *(human sequence, synthetic)*

UbiQ code	:	UbiQ-071
Batch #	:	B01082013-001
Amount	:	50 ug, lyophilized powder
Purity	:	≥95% by RP-HPLC
Mol. Weight	:	9.1 kDa
Storage	:	upon arrival, powder at −20°C; solution at −80°C. Please store dark and avoid multiple freeze/thaw cycles.

Productsheet

Background. UbiQ-071 is an activity-based probe for deubiquitinating enzymes (DUBs). It is based on ubiquitin functionalised with a C-terminal electrophilic vinyl methyl ester (VME) and N-terminal Cy5 dye (exc 625-650 nm, abs 670 nm). It can be used for activity profiling experiments and the control of DUB inhibitor specificity. Whereas the first-generation probes required immunoblotting for detection, the second-generation fluorescent probes allow detection of DUB labeling by in-gel fluorescence. This direct and more sensitive read-out gives more distinct labeling patterns than immunoblotting. In addition, cross-reactivity of antibodies can lead to background labeling, something that is not observed with UbiQ-071.

sequence

Cy5-MQIFVKTLTGKTITLEVEPSDTIENVKAKIQDKEGIPPDQQRLIFAGKQLEDGRTLSDYNIQKESTLHLVLRLRG-**VME**

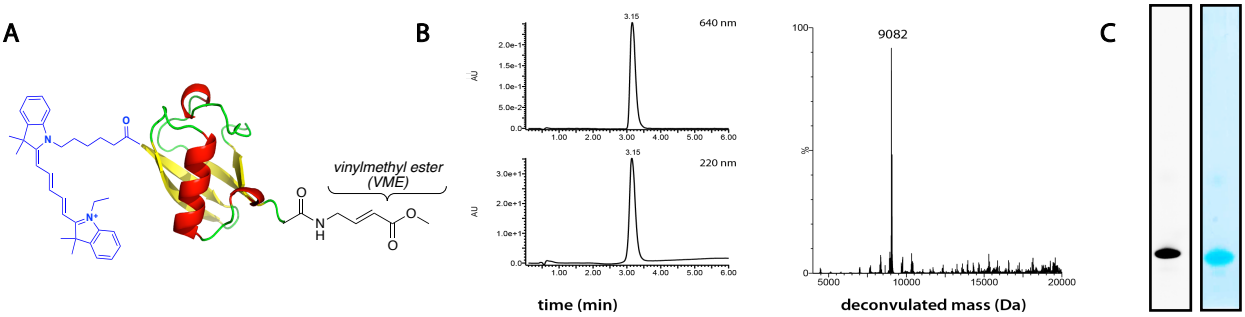

Figure 1. A: UbiQ-071. B: LC-MS analysis. Mobile phase A= 1% CH_3CN, 0.1% formic acid in milliQ and B= 1% milliQ and 0.1% formic acid in CH_3CN. Phenomenex Kinetex C18, (2.1×50 mm, 2.6 µM); flow rate = 0.5 mL/min, column T = 40°C. Gradient: 5-95%B over 3.5 min. C: SDS-PAGE analysis. 12% gel, MES buffer. Left: fluorescence scanning (650/690 nm), right: CBB staining.

important: sample preparation

- dissolve the powder in as little DMSO as possible (e.g., 20 mg/mL)
- add this DMSO stock slowly to milliQ (please note the order of addition) and mix
- for proper folding we advise to buffer this aqueous stock first to 50 mM sodium acetate pH 4.5
- next buffer as desired. For example:
  - dissolve 50 ug probe in 2.5 uL DMSO (20 mg/mL)
  - option 1: add to 47 uL water followed by addition of 0.5 uL 5M NaOAc pH 4.5 to prepare a 1 mg/mL stock in 50 mM NaOAc pH 4.5; this stock is useful when working with low concentrations of probe
  - option 2: add to 45 uL water followed by addition of 2.5 uL 1M HEPES or Tris to prepare a 1 mg/mL stock in 50 mM HEPES/Tris; this stock is useful when working with high concentrations of probe
- For detailed experimental conditions please see open-access reference 1.

Please note we and others have observed the appearance of smearing during SDS-PAGE analysis of (di)Ub conjugates. This can be caused by (heat-induced) aggregation (Morimoto et al. Sci Rep **2018**, 8, article 2711). If possible, avoid heating the samples in Laemmli sample buffer and/or add 4M urea to the SDS-PAGE samples.

Literature. (1) de Jong et al. *ChemBioChem* **2012**, *13*, 2251. (2) Altun et al. *Chem Biol* **2011**, *18*, 1401.

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