UbiQ
targeting the ubiquitin system

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

UbiQ code	:	UbiQ-050
Batch #	:	B01072013-001
Amount	:	50 ug, lyophilized powder
Purity	:	≥95% by RP-HPLC
Mol. Weight	:	9.02 kDa
Storage	:	upon arrival, powder at −20°C, solution at −80°C. Protect from light and avoid multiple freeze/thaw cycles.

Productsheet

Background. UbiQ-050 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 5-carboxytetramethylrhodamine (TAMRA, exc 550 nm, emi 590 nm) dye. 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-050.

sequence

TAMRA-MQIFVKTLTGKTITLEVEPSDTIENVKAKIQDKEGIPPDQQRLIFAGKQLEDGRTLSDYNIQKESTLHLVLRLRG-**VME**

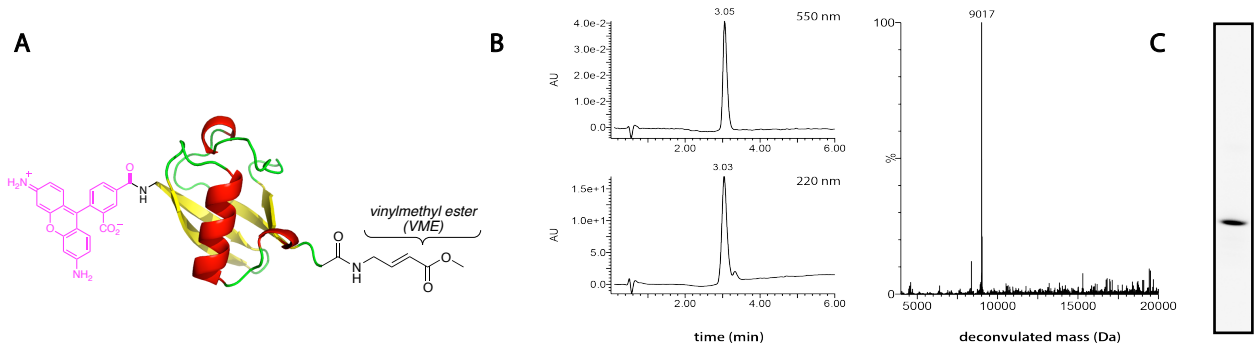

Figure 1. A: LC-MS analysis. Mobile phase A= 1% CH_3CN, 0.1% formic acid in milliQ and B= 1% milliQ and 0.1% formic acid in CH_3CN. Phenomenex Kinetex C18, (2.1×50 mm, 2.6 µM); flow rate= 0.5 mL/min, column T = 40°C. Gradient: 5-95% over 3.5 min. B: SDS-PAGE analysis, 12% gel, MES buffer. Fluorescence scan exc 550 nm, emi 590 nm.

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).
- to ensure proper folding, we advise to first buffer to 50 mM NaOAc pH 4.5 before buffering to a pH >7.
- next, buffer as desired.
- 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 for SDS-PAGE analysis.

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

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