

UbiQ

targeting the ubiquitin system

TAMRA-Ub-VME (human sequence, synthetic)

UbiQ code : UbiQ-050
Batch # : B01072013-001
Amount : 50 ug, lyophilized powder
Purity : $\geq 95\%$ by RP-HPLC and SDS-PAGE analysis
Mol. Weight : 9017 Da by MS (calc Mw 9017 Da)
Storage : upon arrival powder at -20°C , solution at -80°C . Protect from light and avoid multiple freeze/thaw cycles.

Productsheet

Background. TAMRA-Ub-VME (**UbiQ-050**) is a potent, irreversible and specific inhibitor of deubiquitinating enzymes (DUBs),¹ which is labeled on the *N*-terminus with a 5-carboxytetramethylrhodamine (TAMRA, exc 550 nm, emi 590 nm) dye.² This ubiquitin-based activity probe can be used for activity profiling experiments and the control of DUB inhibitor specificity.^{2,3} Whereas the first-generation activity probes (such as HA tagged Ub-VME) require immunoblotting for detection, the second-generation TAMRA-Ub-VME probe allows detection of DUB labeling by in-gel fluorescence (Fig. 2). 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-MQIFVKLTGKTITLEVEPSDTIENVKAKIQDKEGIPPDQQLIFAGKQLEDGRTLSDYNIQKESTLHLVLRRLRG-**VME**

Important: sample preparation

- dissolve the powder in as little DMSO as possible (20 - 40 mg/mL)
- add the DMSO stock to milliQ (please note the order of addition) and mix
- buffer the aq. solution as desired (using 1M HEPES or 1M Tris for example)
- in general, DMSO concentrations up to 5 vol% are well tolerated by most enzymes.
- total removal of DMSO can be accomplished by dialysis or spin-filtration (3 kDa cut-off membrane).

UbiQ

targeting the ubiquitin system

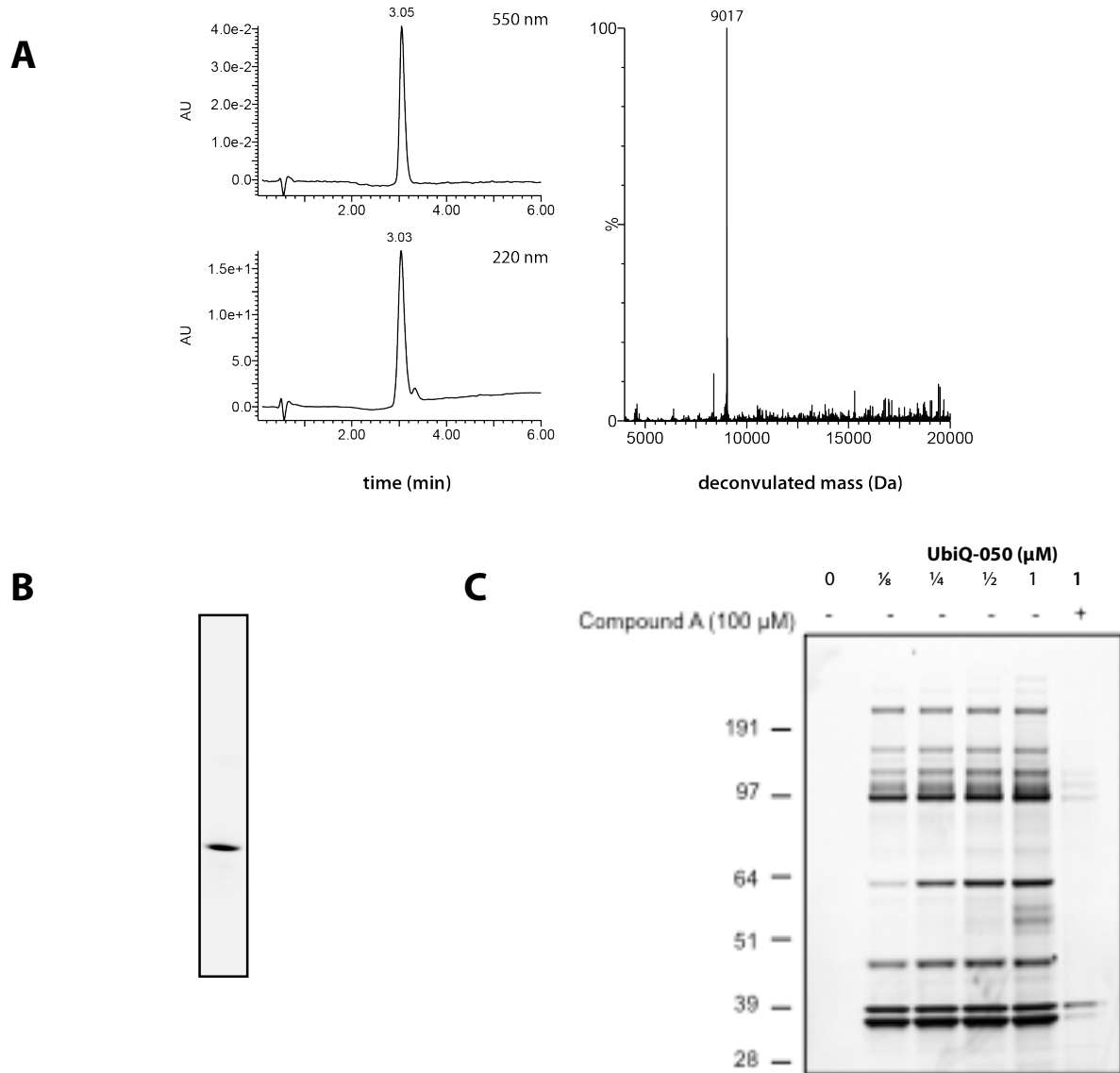


Figure 1. A; LC-MS analysis. Mobile phase A= 1% CH₃CN, 0.1% formic acid in milliQ and B= 1% milliQ and 0.1% formic acid in CH₃CN. Phenomenex Kinetex C18, (2.1×50 mm, 2.6 μ M); flow rate = 0.5 mL/min, column T = 40°C. Gradient: 5% \Rightarrow 95% over 3.5 min. B: SDS-PAGE analysis, 12% gel, MES buffer. Fluorescence scan exc 550 nm, emi 590 nm. C: EL4 lysate was incubated with indicated concentrations of UbiQ-050 at ambient temperature for 15 min. Compound A is a (pan-)DUB inhibitor that was included to show how TAMRA-Ub-VME can be used to monitor DUB inhibitor specificity. In-gel fluorescence scans were obtained by using a ProXPRESS 2D Proteomic imaging system (Perkin-Elmer) with a resolution of 100 μ m and exposure time of 60s, with filter settings ($\lambda_{ex}/\lambda_{em}$) 550/ 590 nm.

Literature. (1) Misaghi et al. *J. Biol. Chem.* **2005**, *280*, 1512. (2) de Jong et al. *ChemBioChem* **2012**, *13*, 2251. (3) Altun et al. *Chem. Biol.* **2011**, *18*, 1401.