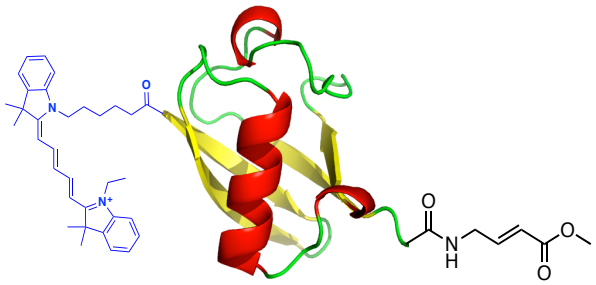
UbiQ

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; buffered solution at –80°C. Please protect from light and avoid multiple freeze/thaw cycles.

Productsheet

Background. Cy5-Ub-VME (**UbiQ-071**) is a potent, irreversible and specific inhibitor of deubiquitinating enzymes (DUBs),[1] which is labeled on the *N*-terminus with a Cy5 dye (Cy5, exc 625-650 nm, abs 670 nm).[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, **UbiQ-071** allows for detection of DUB labeling by in-gel fluorescence. This direct and more sensitive read-out gives more distinct labeling paterns than can be achieved by immunoblotting. In addition, cross-reactivity of antibodies can lead to background labeling, something that is not observed with **UbiQ-071**.

Sequence

Cy5-MQIFVKTLTGKTITLEVEPSDTIENVKAKIQDKEGIPPDQQRLIFAGKQLEDGRTLSDYNIQKESTLHLVLRLRG-**VME**

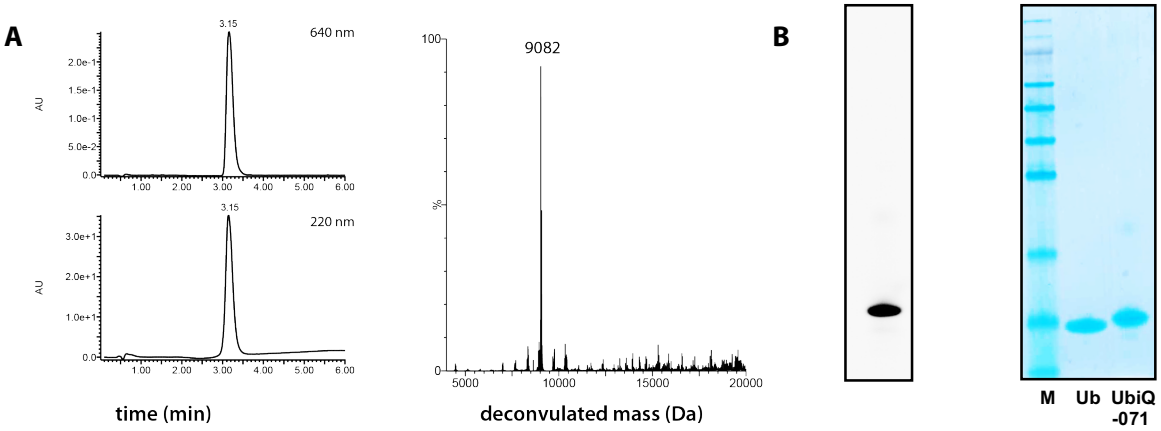


Figure 1. A; LC-MS analysis. Mobile phase A= 1% CH_3CN, 0.1% formic acid in milliQ and B= 1% milliQ and 0.1% formic acid in CH_3CN. Phenomenex Kinetex C18, (2.1×50 mm, 2.6 µM); flow rate = 0.5 mL/min, column T = 40°C. Gradient: 5% ⇨ 95% over 3.5 min. **B: SDS-PAGE analysis**, 12% gel, MES buffer. Left: 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).**
- **buffer as desired (with e.g. 1M HEPES to 50 mM HEPES).**
- **a final buffered stock of for example 0.5 mg/mL contains 2.5 vol% DMSO; in general DMSO concentrations of up to 5 vol% are well tolerated by DUBs.**
- **if required, total removal of DMSO is accomplished by dialysis or spin-filtration (3 kDa cut-off membrane).**

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.

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