

UbiQ

targeting the ubiquitin system

TAMRA-Ub-PA (human sequence, synthetic, alternative name = TAMRA-Ub-Prg)

UbiQ code : UbiQ-058

Batch # : B01082013-001

Amount : bulk, lyophilized powder

Purity : ≥95% by RP-HPLC

Mol. Weight: 8.96 kDa

Storage : upon arrival, powder at -20°C; solution at -80°C. Store dark and avoid multiple freeze/thaw cycles.

Productsheet

Background. UbiQ-058 is an activity-based probe for deubiquitinating enzymes (DUBs). It is based on ubiquitin functionalised with a C-terminal propargyl amide (PA) and N-terminal 5-carboxytetramethylrhodamine (TAMRA) dye (Figure 1A). UbiQ-058 can be used for activity profiling experiments and determining DUB inhibitor specificity. It has three unique capabilities: first, it forms a covalent linkage with (the active site Cys residue of) a DUB that can be cleaved by acid treatment (5% aq. TFA), allowing for proteomic analyses; secondly, it targets all three major DUB families: UCH, USP and OTU; thirdly, the TAMRA label allows detection of DUB labeling by direct in-gel fluorescence (Figure 1C). This is a less time-consuming and more sensitive read-out than western blotting. Finally, cross-reactivity of antibodies can lead to background labeling, something that is not observed with UbiQ-058. Although Ub-PA probes mainly target DUBs, the active-site Cys residue of certain HECT E3 ligases (HUWE1 and NEDD4) has been found to react with Ub-PA (Nair et al.). Therefore, Ub-PA probes can also be used to study certain HECT E3 ligases.

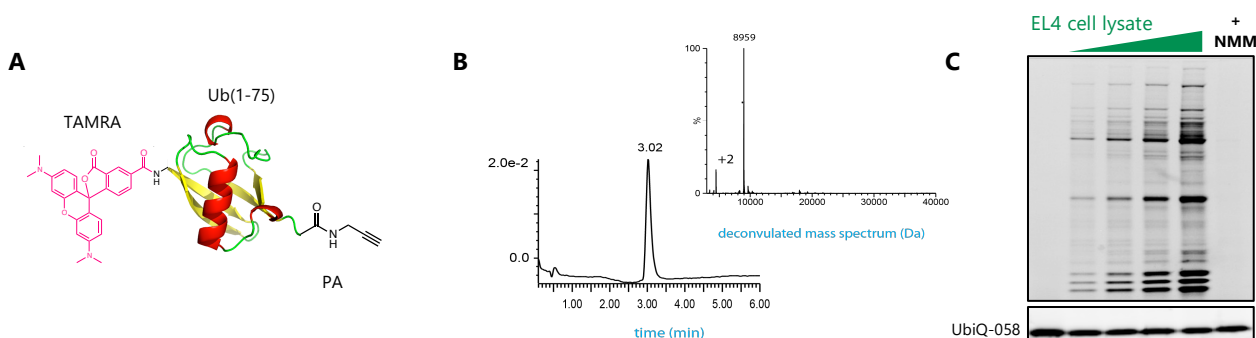


Figure 2. A: UbiQ-058. B: LC-MS analysis. Mobile phase A= 1% CH₃CN, 0.1% formic acid in milliQ and B= 1% milliQ, 0.1% formic acid in CH₃CN. Phenomenex Kinetex C18 (2.1 × 50 mm, 2.6 μm); flow rate= 0.5 mL/min, column T= 40°C. Gradient: 5-95%B over 3.5 min. C: labeling of increasing amount of EL4 lysate with UbiQ-058, NMM= N-methylmaleimide. Fluorescence scan exc/em 550/590 nm.

* Please note smearing can be observed 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 loading buffer for SDS-PAGE analysis or add 4M urea to the loading buffer.

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:
 - ⇒ option 1: dissolve 50 μg probe in 2.5 μL DMSO (20 mg/mL) and add to 47 μL water followed by addition of 0.5 μL 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: dissolve 50 μg probe in 2.5 μL DMSO (20 mg/mL) and add to 45 μL water followed by addition of 2.5 μL 1M HEPES to prepare a 1 mg/mL stock in 50 mM HEPES; this stock is useful when working with high concentrations of probe

Literature. (1) Ekkebus et al. *J Am Chem Soc* **2013**, 135, 2867. (2) Sommer et al. *Bioorg Med Chem* **2013**, 21, 2511. (3) Nair et al. *ACS Chem Biol* **2021**, 16, 1615.

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