UbiQ
targeting the ubiquitin system

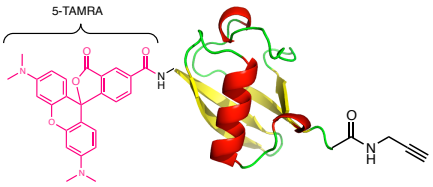


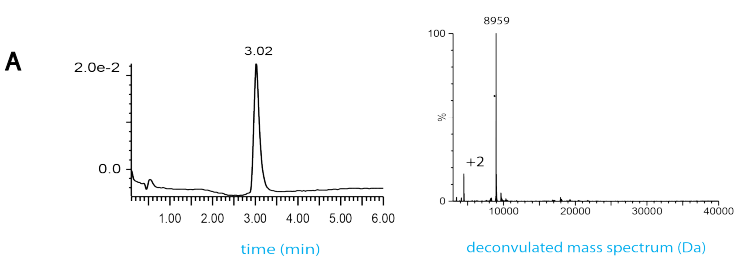
Figure 1

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

UbiQ code	:	UbiQ-058
Batch #	:	B01082013-001
Amount	:	50 ug, 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. 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 2B). 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 based 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; as such Ub-PA based probes can also be used and evaluated for the study of HECT E3 ligases. For more details, please see reference 3 (Nair et al.).



C

TAMRA-MQIFVKTLTGKTITLEVEPSDTIENVKAKIQDKEGIPPDQQRLI
FAGKQLEDGRTLSDYNIQKESTLHLVLRLRG-**PA**

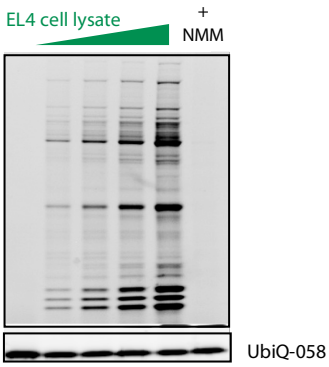


Figure 2. A: LC-MS analysis. Mobile phase A= 1% CH_3CN, 0.1% formic acid in milliQ and B= 1% milliQ, 0.1% formic acid in CH_3CN. Phenomenex Kinetex C18, (2.1×50 mm, 2.6 µM); flow rate= 0.5 mL/min, column T= 40°C. Gradient: 5-95% over 3.5 min. B: labeling of increasing amount of EL4 lysate with UbiQ-058, NMM= N-methylmaleimide. Fluorescence scan exc 550 nm, emi 590 nm. C: sequence.

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
- full experimental details can be found in 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) 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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