

UbiQ

targeting the ubiquitin system

Cy5-Ub-PA (human sequence, synthetic, alternative name =Cy5-Ub-Prg)

UbiQ code : UbiQ-072

Batch # : B01082013-001

Amount : 50 ug, lyophilized powder

Purity : ≥95% by RP-HPLC

Mol. Weight : 9.0 kDa

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

Productsheet

Background. UbiQ-072 is an activity-based probe for deubiquitinating enzymes (DUBs). It is labeled on the *N*-terminus with a Cy5 dye (exc 625-650 nm, emi 670 nm) and a propargyl amide (PA) on the C-terminus. UbiQ-072 can be used for activity profiling experiments and determining DUB inhibitor specificity. The PA group 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 Cy5 label allows detection by direct in-gel fluorescence. 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-072. 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 the PA group. As such PA based probes can also be used and evaluated for the study of HECT E3 ligases. For more details, please see reference 5 (Nair et al.).

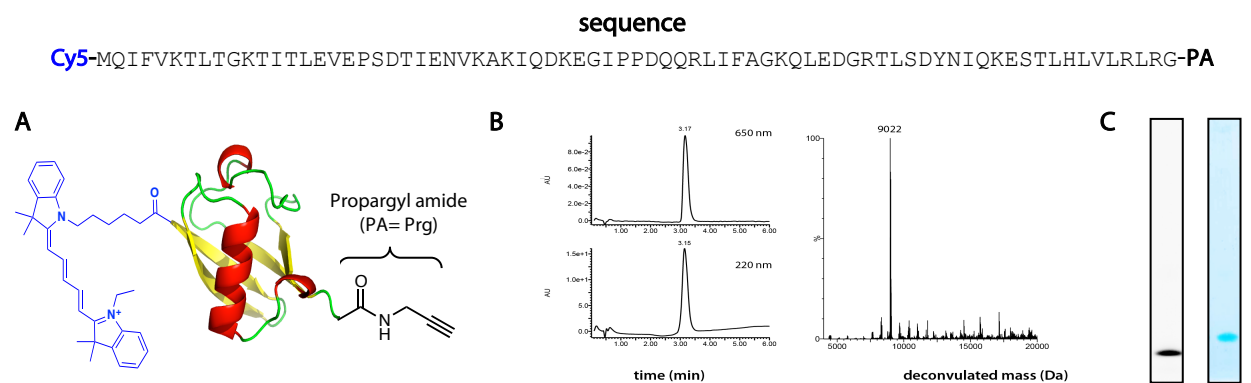


Figure 1. A: UbiQ-072. B: LC-MS analysis. Mobile phase A= 1% CH₃CN, 0.1% formic acid in milliQ and B= 1% milliQ and 0.1% formic acid in CH₃CN. Phenomenex Kinetex C18, (2.1×50 mm, 2.6 μM); flow rate = 0.5 mL/min, column T = 40°C. Gradient: 5-95%B over 3.5 min. B: SDS-PAGE analysis, 12% gel, MES buffer. 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) 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 for SDS-PAGE analysis 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) de Jong et al. *ChemBioChem* **2012**, *13*, 2251. (4) Altun et al. *Chem Biol* **2011**, *18*, 1401. (5) Nair et al. *ACS Chem Biol* **2021**, *16*, 1615.