

UbiQ

targeting the ubiquitin system

Cy5-Ub-PA (human sequence, synthetic)

UbiQ code : UbiQ-072

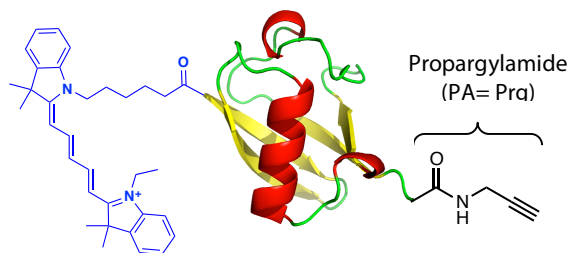
Batch # : B01082013-001

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 in dark environment and avoid multiple freeze/thaw cycles.



Productsheet

Background. Cy5-Ub-PA (**UbiQ-072**) is a potent and specific inhibitor of deubiquitinating enzymes (DUBs) containing the new electrophilic propargylamide (PA, also sometimes abbreviated as Prg).^{1,2} This activity probe is labeled on the N-terminus with a Cyanine5 dye (Cy5, exc 625-650 nm, emi 670 nm) and can be used for activity profiling experiments and determining DUB inhibitor specificity.^{3,4} Due to the PA group, 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 (Fig 1A);¹ secondly, it targets all three major DUB families: UCH, USP and OTU;³ thirdly, the TAMRA label allows detection of DUB labeling by direct in-gel fluorescence (Figure 1).³ 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**.

Sequence

Cy5-MQIFVKLTGKTITLEVEPSDTIENVKAKIQDKEGIPDPQRLIFAGKQLEDGRTLSDYNIQKESTLHLVLRG-PA

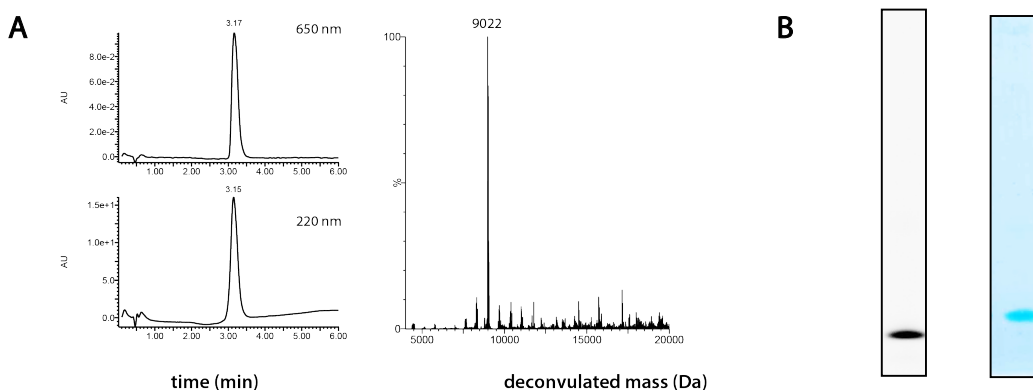


Figure 1. A; LC-MS analysis. Mobile phase A= 1% CH₃CN, 0.1% formic acid in milliQ and B= 1% milliQ and 0.1% formic acid in CH₃CN. Phenomenex Kinetex C18, (2.1×50 mm, 2.6 μm); flow rate = 0.5 mL/min, column T = 40°C. Gradient: 5% ⇒ 95% over 3.5 min. **B: SDS-PAGE analysis**, 12% gel, MES buffer. Left: fluorescence scanning (650/690 nm), right: CBB staining.

Important: sample preparation

- dissolve the powder in as little DMSO as possible (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.
- For detailed experimental conditions please see the open-access references 1 and 3:
- <http://pubs.acs.org/doi/abs/10.1021/ja309802n>
- <http://onlinelibrary.wiley.com/doi/10.1002/cbic.201200497/abstract>

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.