UbiQ
targeting the ubiquitin system

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

UbiQ code	:	UbiQ-057
Batch	:	B01042020-001
Amount	:	50 ug, lyophilized powder
Purity	:	≥95% by RP-HPLC
Mol. Weight	:	8.545 Da
Storage	:	upon arrival, store powder at −20°C; buffered solution at −80°C. Please avoid multiple freeze/thaw cycles.

Productsheet

Background. UbiQ-057 is an activity-based probe for deubiquitinating enzymes (DUBs). It is based on ubiquitin functionalised with a C-terminal propargyl amide (PA). It can be used for activity profiling experiments and determining DUB inhibitor specificity. UbiQ-057 has two unique characteristics: 1) it forms a covalent linkage with (the active site Cys of) a DUB that can be cleaved by acid treatment (5% aq. TFA, Figure 2A), allowing proteomic analyses; 2) it targets all three major DUB families: UCH, USP and OTU (Figure 2BC). 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.).

sequence

MQIFVKTLTGKTITLEVEPSDTIENVKAKIQDKEGIPPDQQRLIFAGKQLEDGRTLSDYNIQKESTLHLVLRLRG-**PA**

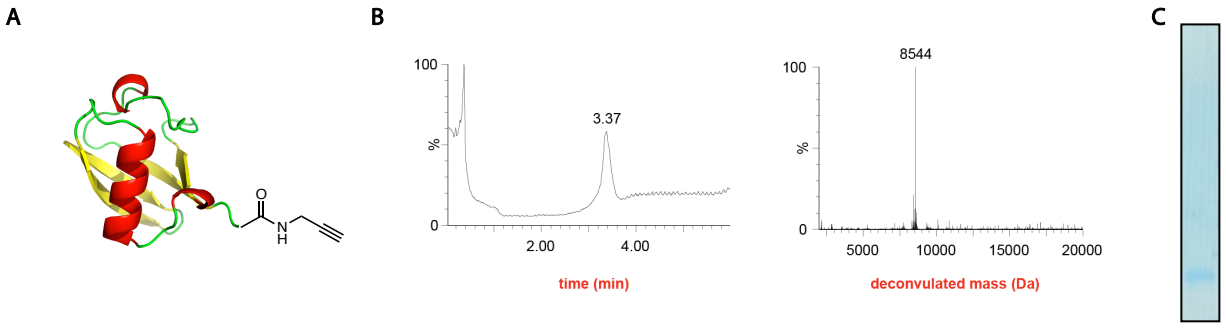

Figure 1. A: Ub-PA structure. B: LC-MS analysis. Mobile phase A= 1% CH_3CN, 0.1% formic acid in milliQ and B= 1% milliQ and 0.1% formic acid in CH_3CN. XBridge BEH300 C18 5µm 4.6x100mm; column T= 40°C, flow= 0.8 mL/min. Gradient: 20–50% over 3.5 min. C: SDS-PAGE analysis. 12% Bolt Bis-Tris Plus gel (Lifetechnologies) in MES buffer, staining with *Instant Blue*.

important: sample preparation

- dissolve the powder in as little DMSO as possible (e.g. 40 mg/mL)
- add this DMSO stock to milliQ (e.g. diluting 40× to 1 mg/mL= 117 uM, 2.5 vol% DMSO)
- buffer as desired.
- full experimental details can be found in reference 1.

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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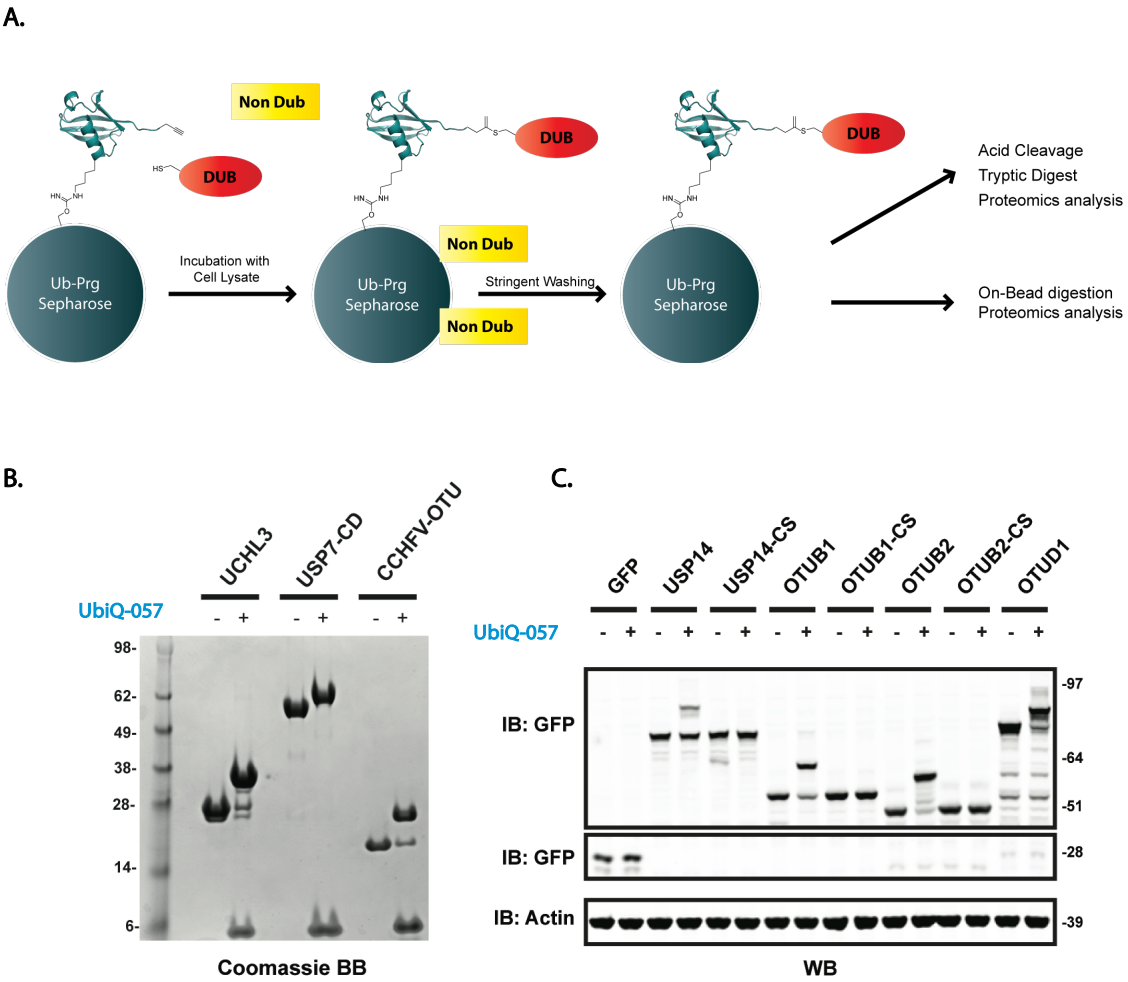

Figure 2. A. Overview of Click-on/Click-off pull down. Ub-Prg can be directly immobilized onto Sepharose resin. The immobilized probe is incubated with a mixture of DUBs and non-DUBs (i.e. lysate). Cysteine DUBs will selectively react with immobilized UbiQ-057, resulting in their covalent attachment. Stringent washing removes unbound non-DUBs. After purification, the DUBs can be cleaved under radical conditions for retrieval of active DUBs or by treatment with 5% aq. trifluoroacetic acid for MS-profiling. B: SDS-PAGE analysis of in vitro reaction of three different classes of DUBs with UbiQ-057. C: GFP fusions of DUBs from the USP and OTU-classes were transfected in MelJuSo cells and their reaction with UbiQ-057 visualized using anti-GFP western blot. DUBs annotated with –CS are catalytic Cys to Ser mutants. Experimental procedures for UbiQ-057 can be found in the *open-access* reference 1.