

UbiQ

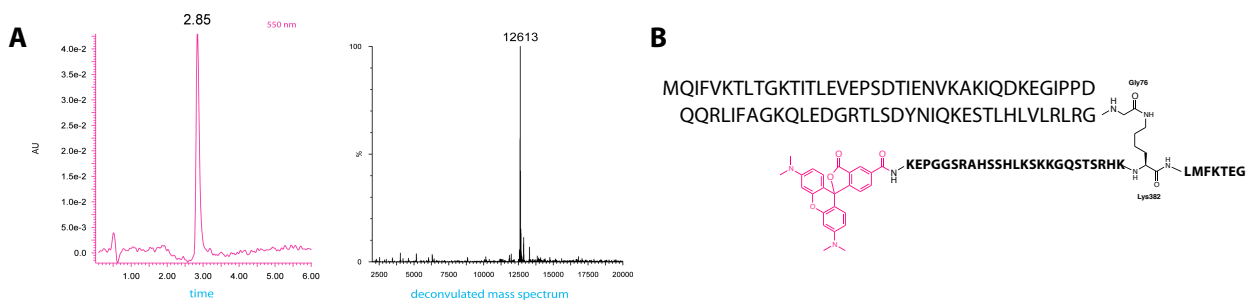
targeting the ubiquitin system

K382 Ub-p53(357-389)-FP

UbiQ code : UbiQ-042
Batch # : B09082012-001
Amount : 25 ug, lyophilized powder
Purity : $\geq 95\%$ by RP-HPLC
Mol. Weight : 12613 Da by MS (calc 12619 Da)
Storage : powder at -20°C ; solution at -80°C . Protect from light and avoid multiple freeze/thaw cycles.

Productsheet

Background. Class II fluorescence polarization HTS reagent¹⁻⁴ based on the C-terminal peptide sequence 357 – 389 of p53, which contains various lysine residues that are (mono)ubiquitinated. The peptide is modified on the N-terminus with a 5-carboxytetramethylrhodamine and conjugated at Lys382 to Ub via a native isopeptide bond. UbiQ-042 contains a PGGs motif (aa 359-362) which was found to be important for USP7 binding.⁷ See reference 5 (open access) for full experimental and analytical details.



A: LC-MS analysis. Mobile phase A = 1% CH_3CN , 0.1% formic acid in water (milliQ) and B = 1% water (milliQ) and 0.1% formic acid in CH_3CN . Phenomenex Kinetex C18, (2.1x50 mm, 2.6 μM); flow rate = 0.5 mL/min, runtime = 6 min, column T = 40°C . Gradient: 5% \Rightarrow 95% over 3.5 min. **B:** Sequence UbiQ-042

Important: sample preparation

- prepare (for example) a 2 mM DMSO stock (23 mg/mL)
- add the DMSO stock to milliQ, e.g. a 2 mM DMSO stock diluted 20x in milliQ affords a 100 μM stock which can be aliquoted and stored.
- for assays this 100 μM stock can be diluted for example 1000x in buffer affording a final assay solution of 100 nM. The DMSO concentration during the assay is now 0.01 vol%.
- the concentration of UbiQ-030 can be verified by comparing the fluorescence intensity with that of a known concentration of TAMRA.
- In general, DMSO conc up to 5 vol% are well tolerated by most DUBs

Literature. (1) Tirat et al. *Anal. Biochem.* **2005**, 343, 244. (2) Huang et al. *Methods in Molecular Biology* **2009**, 565, 127. (3) Levine et al. *Anal. Biochem.* **1997**, 247, 83. (4) Faesen et al. *Chem. Biol.* **2011**, 18, 1550. (5) Geurink et al. *ChemBiochem* **2012**, 13, 293. (6) Mevissen et al. *Cell* **2013**, 154, 169. (7) Sheng et al. *Nat. Struct. Mol. Biol.* **2006**, 13, 285.