UbiQ
targeting the ubiquitin system

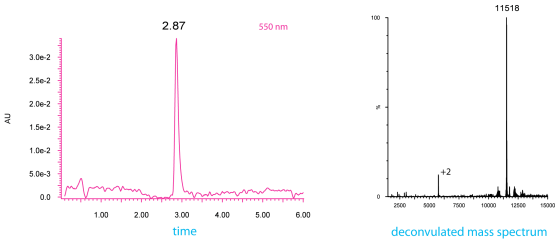
K382 Ub-p53(368-389)-FP

UbiQ code	:	UbiQ-041
Batch #	:	B09082012-001
Amount	:	25 ug, lyophilized powder
Purity	:	≥95% by RP-HPLC
Mol. Weight	:	11518 Da by MS (calc 11523 Da)
Storage	:	powder at –20°C; solution at –80°C. Protect from light and avoid multiple freeze/thaw cycles.

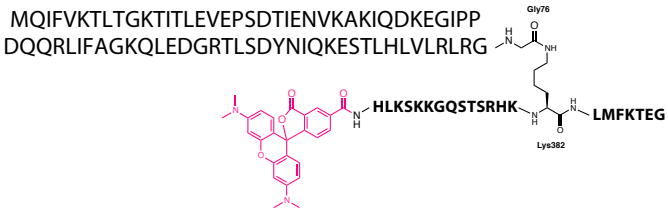
Productsheet

Background. Class II fluorescence polarization HTS reagent based on the C-terminal peptide sequence 368 – 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. See reference 5 (open access) for full experimental and analytical details.

A



B



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, (2.1×50 mm, 2.6 µM); flow rate = 0.5 mL/min, runtime = 6 min, column T = 40°C. Gradient: 5% ⇨ 95% over 3.5 min. **B:** Sequence UbiQ-041

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 20× in milliQ affords a 100 µM stock which can be aliquoted and stored.**
- **for assays this 100 µM stock can be diluted for example 1000× 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.

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