

Ub-FANCD2(557-565)-FP (human sequence, synthetic)

UbiQ code : UbiQ-029
 Batch # : B10102013-001
 Amount : 25 ug, lyophilized powder
 Purity : ≥95% by RP-HPLC
 Mol. Weight : 10.0 kDa
 Storage : upon arrival, powder at –20°C; solution at –80°C. Store dark and avoid multiple freeze/thaw cycles.

Productsheet

Background. UbiQ-029 is a class II fluorescence polarization HTS reagent based on the peptide sequence 557 – 565 of FANCD2, a DNA repair protein which is monoubiquitinated on Lys561. The peptide is modified on the N-terminus with a 5-carboxytetramethylrhodamine (5-TAMRA) dye and conjugated at Lys561 to ubiquitin via a native isopeptide bond. Typical substrate concentrations range from 10 – 100 nM. DUB concentrations can range from 0.01 – 10 nM but depend on specific assay conditions and method of detection.

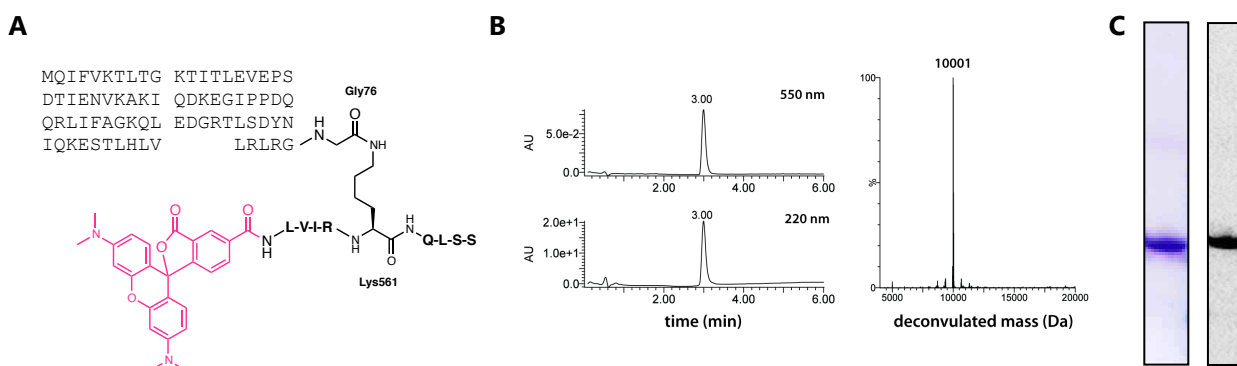


Figure 1. A: sequence UbiQ-029. B: LC-MS analysis. Mobile phase A= 1% CH₃CN, 0.1% formic acid in water and B= 1% water and 0.1% formic acid in CH₃CN. Phenomenex Kinetex C18, (2.1×50 mm, 2.6 μM); flow rate = 0.8 mL/min, column T = 40°C. Gradient: 5-95%B over 3.5 min. C: SDS-PAGE analysis. 12% SDS-PAGE gel. Left gel: Coomassie Brilliant Blue staining. Right gel: fluorescence scan (exc 550 nm, emi 590 nm).

important: sample preparation

- dissolve the powder in DMSO (e.g., 1 mg/mL= 100 uM)
- 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)
- final assay stocks of 100 nM will contain 0.1 vol% DMSO when prepared from a 100 uM DMSO stock
- for assays requiring higher UbiQ-029 concentrations, prepare a more concentrated DMSO stock (e.g., 10 - 25 mg/mL)
- full exp. details can be found in open-access reference 4: Geurink et al. *ChemBiochem*, **2012**, 13, 293.

Literature. (1) Tirat et al. *Analytical Biochem* **2005**, 343, 244. (2) Huang et al. *Methods Mol Biol* **2009**, 565, 127. (3) Levine et al. *Analyt Biochem* **1997**, 247, 83. (4) Geurink et al. *ChemBiochem* **2012**, 13, 293.