

Ub-Ub(56-69)-FP K63 linked (human sequence, synthetic)

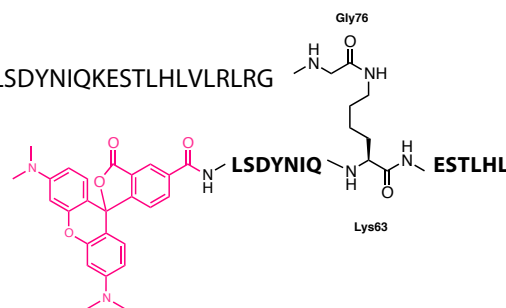
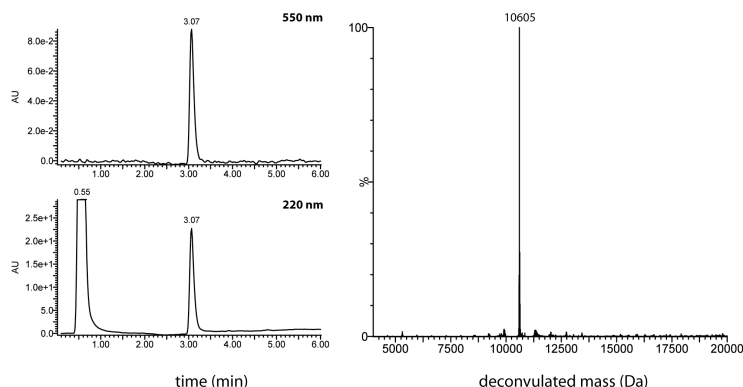
UbiQ code : UbiQ-049
 Batch # : B01012013-001
 Amount : 1.2 ul of 2 mM DMSO stock (21 ug/ul)= 25 ug
 Purity : ≥95% by RP-HPLC and SDS-PAGE
 Mol. Weight : 10.6 kDa
 Storage : upon arrival powder at -20°C; solution at -80°C. Protect from light and avoid multiple freeze/thaw cycles.

Productsheet

Background. UbiQ-049 is a class II fluorescence polarization HTS reagent¹⁻³ based on peptide sequence 56–69 of ubiquitin.⁴ The peptide is modified on the N-terminus with a 5-carboxytetramethylrhodamine and conjugated at Lys63 to Ub via a native isopeptide bond. See references 4-6 for full experimental and analytical details.

Sequence

MQIFVKLTGKITLEVEPSDTIENVKAKIQDKEGIPDPQRLIFAGKQLEDGRTLSDYNIQKESTLHLVLRRLRG



LC-MS analysis. 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% B over 3.5 min. Mobile phase A = 1% CH₃CN, 0.1% formic acid in water (milliQ) and B = 1% water (milliQ) and 0.1% formic acid in CH₃CN.

Important: sample preparation

- dilute the 2 mM DMSO stock (e.g.) 20× in milliQ affording a 100 μM stock
- 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-049 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
- all stocks can be aliquoted and stored.

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.