

Ub-Ub(4-17)-FP K11 linked (human sequence, synthetic)

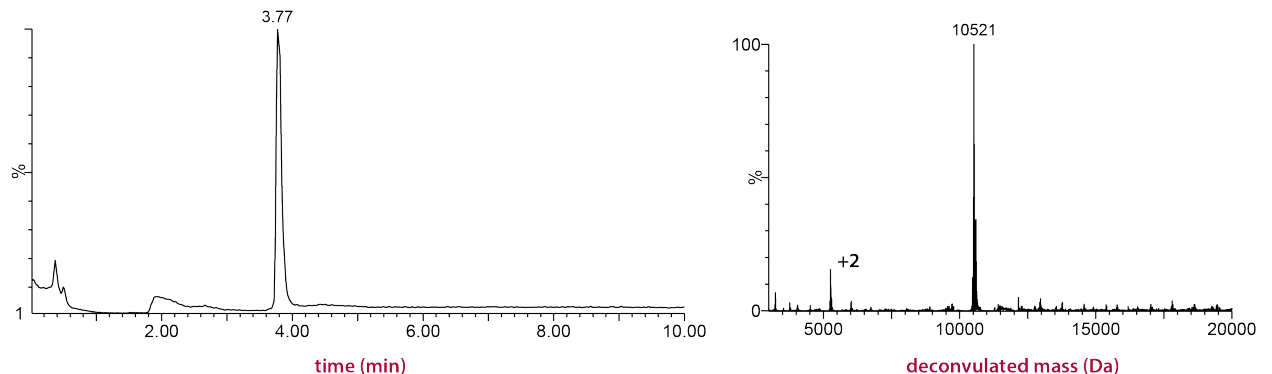
UbiQ code : UbiQ-044
Batch # : B15102014-001
Amount : 25 ug, solution in DMSO (1 uL, 25 mg/mL, 2.38 mM)
Purity : $\geq 95\%$ by RP-HPLC
Mol. Weight : 10.5 kDa
Storage : upon arrival store at -80°C . Protect from light. Please avoid multiple freeze/thaw cycles.

Productsheet

Background. Class II fluorescence polarization HTS reagent¹⁻³ based on the peptide sequence 4–17 of ubiquitin.⁴ The peptide is modified on the *N*-terminus with a 5-carboxytetramethylrhodamine (5-TAMRA) dye and conjugated at Lys11 to Ub via a native isopeptide bond. See references 4–6 for full experimental and analytical details.

Sequence (5-TAMRA)-FVKTLTGK(Ub)TITLVEV

Ub= MQIFVKTLTGKTITLEVEPSDTIENVKAKIQDKEGIPPDQQRLLIFAGKQLEDGRTLSDYNIQKESTLHLVLRLLGG



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 . XSelect CSH C18 (4.6 \times 100 mm, 3.5 μM); flow rate= 0.8 mL/min, runtime = 6 min, column T= 40°C . Gradient: 30% \Rightarrow 60%B over 5.5 min.

Important: sample preparation

- dilute the DMSO stock in milliQ - *please add DMSO to milliQ* - to a 100 μM stock
- for assays, the 100 μM stock can be diluted for example 1000 \times in buffer, affording a final assay solution of 100 nM. The DMSO concentration is now 0.01 vol%.
- the concentration of UbiQ-044 can be verified by comparing the fluorescence intensity with that of a known concentration of 5-TAMRA
- all stocks are suitable for storage

General experimental procedures for fluorescence polarization assays

FP assays were performed on a PerkinElmer Wallac EnVision 2100 Multilabel Reader with a 531 nm excitation filter and two 579 nm emission filters. Fluorescence intensities were measured in the S (parallel) and P (perpendicular) direction. FP values are given in mP (millipolarization) and calculated using the following formula (1):

$$\text{Polarization (mP)} = \frac{S-(G \cdot P)}{S+(G \cdot P)} \cdot 1000 \quad (1)$$

The confocal optics were adjusted with the average P and S values for TAMRA-Lys-Gly and the grating factor (G) was determined using a polarization value (L) for TAMRA of 50 mP using the following formula (2):

$$G = \frac{\text{average S}}{\text{average P}} \cdot \frac{1 - \left(\frac{L}{1000}\right)}{1 + \left(\frac{L}{1000}\right)} \quad (2)$$

The assays were performed in “non binding surface flat bottom low flange” black 384-well plates (Corning) at room temperature in a buffer containing 20 mM Tris-HCl, pH 7.5, 5 mM DTT, 100 mM NaCl, 1 mg/mL 3-[(3-cholamidopropyl) dimethylammonio] propanesulfonic acid (CHAPS) and 0.5 mg/mL bovine gamma globulin (BGG). Each well had a volume of 20 μ L. Buffer and enzyme were predispensed and the reaction was started by the addition of substrate. Kinetic data was collected in intervals of 2.5 or 3 min. From the obtained polarization values (P_t) the amount of processed substrate (S_t) was calculated with to the following formula (3):

$$S_t = S_0 - S_0 \times \left[\frac{P_t - P_{\min}}{P_{\max} - P_{\min}} \right] \quad (3)$$

Where P_t is the polarization measured (in mP); P_{\max} is the polarization of 100% unprocessed substrate (determined for every reagent at all used substrate concentrations); P_{\min} is the polarization of 100% processed substrate; S_0 is the amount of substrate added to the reaction.

From the obtained P_t values the values for initial velocities (v_i) were calculated. The v_i values are used to determine the Michaelis-Menten constants (K_m , V_{\max} and k_{cat}) by fitting the data according to formula (4) (where $k_{\text{cat}} = V_{\max}/[E]$). All experimental data was processed using Ms Excel and Prism 4.03 (GraphPad Software, Inc.).

$$v_i = \frac{V_{\max} \times S_0}{K_m + S_0} \quad (4)$$

Literature. (1) Tirat et al. *Analytical Biochem* **2005**, *343*, 244. (2) Huang et al. *Methods in Molecular Biology* **2009**, *565*, 127. (3) Levine et al. *Analytical Biochem* **1997**, *247*, 83. (4) Faesen et al. *Chem Biol* **2011**, *18*, 1550. (5) Geurink et al. *ChemBiochem* **2012**, *13*, 293. (6) Bingol et al. *Nature* **2014**, *510*, 370.