UbiQ
targeting the ubiquitin system

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, solution at −80°C. Store dark and avoid multiple freeze/thaw cycles.

Productsheet

Background. UbiQ-049 is a class II fluorescence polarization reagent based on peptide sequence 56–69 of ubiquitin. The peptide is modified on the N-terminus with a 5-carboxytetramethylrhodamine (TAMRA) and conjugated at Lys63 to Ub via a native isopeptide bond.

sequence

TAMRA-LSDYNIQK(Ub)ESTLHL

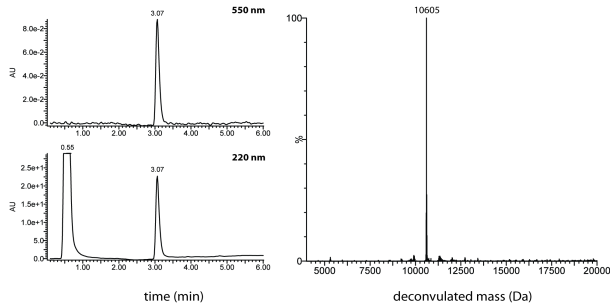

Figure 1. LC-MS analysis. Phenomenex Kinetex C18, (2.1×50 mm), 2.6 µM); flow rate = 0.5 mL/min, column T = 40°C. Gradient: 5-95% B over 3.5 min. Mobile phase A= 1% CH_3CN, 0.1% formic acid in water and B= 1% water and 0.1% formic acid in CH_3CN.

important: sample preparation

- dilute DMSO stock with milliQ and mix well (e.g., 20x to a 100 µM stock; this 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 can be verified by comparing the fluorescence intensity with that of a known concentration of TAMRA.
- for full experimental details please see reference 5.

Literature. (1) Tirat et al. *Analytical Biochem.* **2005**, *343*, 244. (2) Huang et al. *Methods in Mol Biol* **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.

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General experimental procedures for fluorescence polarization assays

FP assays were performed on a PerkinElmer Wallac EnVision 2010 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):

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

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):

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

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 µL. 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):

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

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 $k_{cat} = V_{max}/[E]$). All experimental data was processed using Ms Excel and Prism 4.03 (GraphPad Software, Inc.).

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