

UbiQ

targeting the ubiquitin system

5-TAMRA-Lys(Nedd8)-Gly *(human sequence, semi-synthetic)*

UbiQ code : UbiQ-019

Batch # : B01052014-001

Amount : 50 ug, lyophilized powder

Purity : $\geq 95\%$ by SDS-PAGE analysis

Mol. Weight : 9.16 kDa

Storage : upon arrival, powder at -20°C , solution at -80°C . Protect from light and avoid multiple freeze/thaw cycles.

Productsheet

Background. UbiQ-019 is Class I fluorescence polarization HTS reagent¹⁻³ based on the Lys-Gly dipeptide. The peptide is modified on the N-terminus with a 5-carboxytetramethylrhodamine and mononeddylated on the Lys via a native isopeptide bond. It can be used as a substrate for Nedd8 proteases. This product is formed by chemical ligation.⁴ See reference 5 (open access) and page 2 for standard FP assay details.



SDS-PAGE analysis UbiQ-019. 12% Bolt Bis-Tris Plus gel (Life technologies), MES buffer. M= SeeBlue Plus2 Pre-stained Standard (Invitrogen).

important: sample preparation

- dissolve the powder in DMSO (e.g. $0.92 \text{ mg/mL} = 100 \text{ uM}$ or $9.2 \text{ mg/mL} = 1000 \text{ uM}$)
- **add the DMSO stock to 300 mM NaCl (please note the order of addition) and mix - at this step we have included a high salt aq. solution because Nedd8 is more stable at high salt concentration.**
- 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 example.
- all stocks are suitable for storage at -80°C
- the concentration of the FP reagent can be verified by comparing the fluorescence intensity with that of a known concentration of TAMRA.

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):

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

The confocal optics are adjusted with the average P and S values for TAMRA or TAMRA-Lys-Gly (**UbiQ-023**) and the grating factor (G) was determined using a polarization value (L) for TAMRA of 50 mP using the following formula (2):

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

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 $k_{cat} = V_{max}/[E]$). 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. *Analyt Biochem* **2005**, 343, 244-255. (2) Huang et al. *Methods in Molecular Biology* **2009**, 565, 127. (3) Levine et al. *Analyt 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.