UbiQ
targeting the ubiquitin system

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

UbiQ code	:	UbiQ-019
Batch #	:	B01052014-001
Amount	:	50 ug, lyophilized powder
Purity	:	≥95%
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 (5-TAMRA-Lys(Nedd8)-Gly) is a fluorescence polarization assay reagent for deneddylating enzymes. It is based on a 5-carboxytetramethylrhodamine (TAMRA, exc 550 nm, emi 590 nm) modified Lys-Gly dipeptide that is linked with Nedd8 via a native isopeptide bond (Figure 1A).

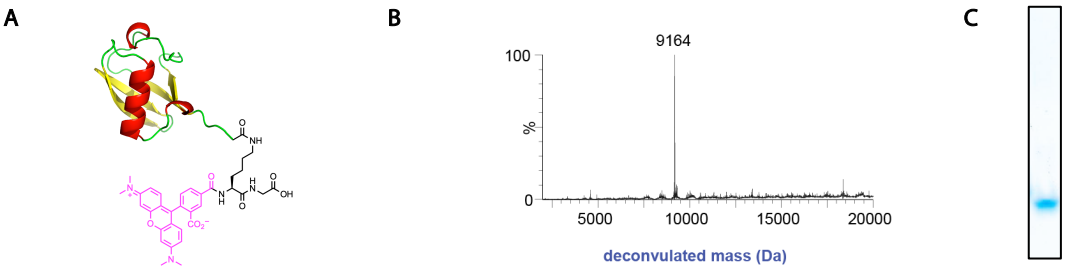

Figure 1. A: UbiQ-019. B: MS analysis. C: SDS-PAGE analysis. 12% Bolt Bis-Tris Plus gel (Life technologies), MES buffer, CBB staining.

important: sample preparation

- dissolve the powder in DMSO (e.g., 0.92 mg/mL= 100 uM or 9.2 mg/mL= 1000 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
- 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
- full exp. details can be found in open-access reference 5: Geurink et al. *ChemBiochem*, **2012**, *13*, 293.

Literature. (1) Tirat et al. *Analytical Biochem* **2005**, *343*, 244-255. (2) Huang et al. *Methods 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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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):

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

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):

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

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_i) the amount of processed substrate (S_t) was calculated with to the following formula (3):

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

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.).

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