

# UbiQ

targeting the ubiquitin system

## K720 Nedd8 cullin-1 (715-725) FP (*human sequence, synthetic*)

UbiQ code : UbiQ-096

Batch # : B01082014-001

Amount : 25 ug, lyophilized powder

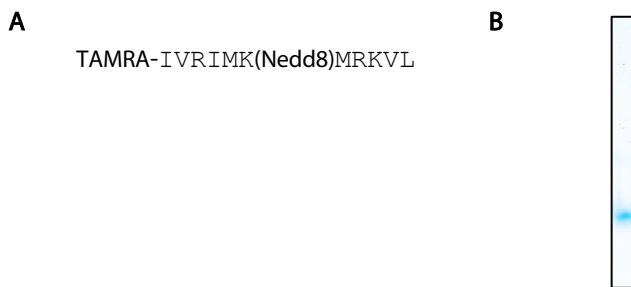
Purity : ≥95%

Mol. Weight : 10.35 kDa

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

## Productsheet

**Background.** UbiQ-096 is a fluorescence polarization reagent based on the cullin-1 (715-725) peptide. The peptide is modified on the N-terminus with a 5-carboxytetramethylrhodamine (TAMRA) and mononeddylated at K720 via a native isopeptide bond. It can be used as a substrate for Nedd8 proteases.



**Figure 1.** Sequence (A) and SDS-PAGE analysis (B). 12% Bolt Bis-Tris Plus gel (Life technologies), MES buffer.

### 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.

## 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):

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

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):

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

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  $\mu$ 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.).

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