UbiQ
targeting the ubiquitin system

K720 Nedd8 cullin-1 (711-730) FP *(human sequence, synthetic)*

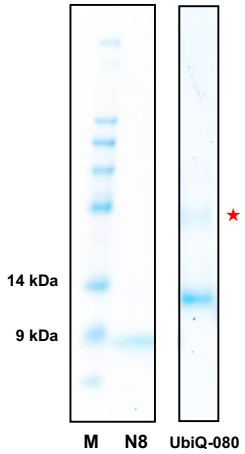
UbiQ code	:	UbiQ-080
Batch #	:	B24072012-001
Amount	:	25 ug, lyophilized powder
Purity	:	≥95% by LC-MS
Mol. Weight	:	11.37 kDa
Storage	:	upon arrival powder at –20°C, solution at –80°C. Protect from light and avoid multiple freeze/thaw cycles.

Productsheet

Background. UbiQ-080 is Class II fluorescence polarization HTS reagent[1-4] based on the cullin-1 (711-730) polypeptide. The peptide is modified on the N-terminus with a 5-carboxytetramethylrhodamine and mononeddylated at K720 via a native isopeptide bond. It can be used as a substrate for Nedd8 proteases.[1,2] This product is formed by chemical ligation.[5] See reference 4 (open access) for full experimental assay details.

Sequence

(5-TAMRA)-IQAAIVRIMK(Nedd8)MRKVLKHQQL



SDS-PAGE analysis. SDS-PAGE analysis. 12% Bolt Bis-Tris Plus gel (Life technologies), MES buffer. M= SeeBlue Plus2 Pre-stained Standard (Invitrogen). ★= aggregation artefact (due to not heating sample prior to SDS-PAGE) – verified by LC-MS

Important: sample preparation

- **dissolve the powder in DMSO (e.g. 1.14 mg/mL= 100 uM or 11.4 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 (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**
- **all stocks are suitable for storage at –80°C**

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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):

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

The confocal optics are adjusted with the average P and S values for TAMRA 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 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):

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

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) A. Faesen et al. *Chem. & Biol.* **2011,** *18*, 1550. (2) Huang et al. *Methods in Molecular Biology* **2009**, *565*, 127. (3) Levine et al. *Anal. Biochem.* **1997**, *247*, 83. (4) Geurink et al. *ChemBiochem*, **2012**, *13*, 293. (5) El Oualid et al. *Angew. Chem. Int. Ed.* **2010**, *49*, 10149.

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