UbiQ
targeting the ubiquitin system

K61 Ub-Tom20(51-65)-FP *(human, synthetic)*

UbiQ code	:	UbiQ-100
Batch #	:	B01102014-001
Amount	:	100 µg, lyophilized powder
Purity	:	≥95% by RP-HPLC
Mol. Weight	:	calc 10.543 Da, found 10.542 Da
Storage	:	upon arrival store powder at −20°C and solution at −80°C. Please protect from light and avoid multiple freeze/thaw cycles.

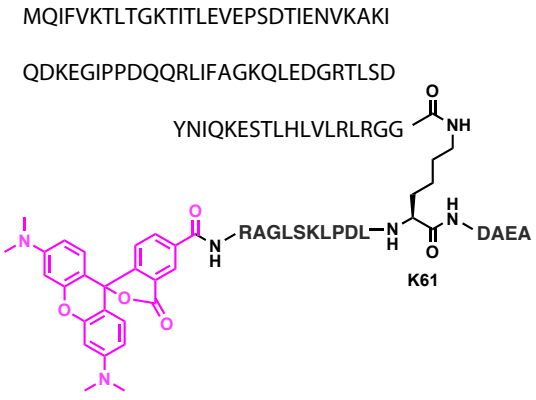
Productsheet

Background. UbiQ-100 is a class II fluorescence polarization HTS reagent[1-5] based on the peptide sequence 51 – 61 of Tom20, a mitochondrial protein which has recently been identified as a substrate for USP30.[6] The Tom20 peptide is modified on the N-terminus with a 5-carboxytetramethylrhodamine and conjugated at Lys61 to ubiquitin (Ub) via a native isopeptide bond. See reference 5 (open access) and page 2 for FP assay details.

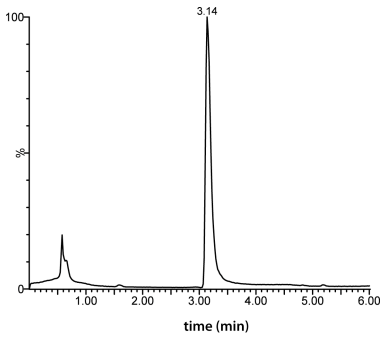
Important: sample preparation.

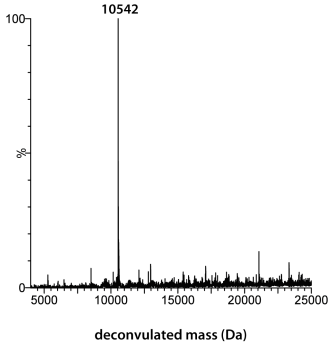
- dissolve the powder in DMSO to a stock of e.g. 1000 uM
- = 2.4 uL DMSO/25 ug of **UbiQ-100**
- dilute the DMSO stock 10× in milliQ - **please add DMSO to milliQ**
- this 100 µM stock can be diluted e.g. 1000× in buffer affording a final (standard) 100 nM assay solution.
- the DMSO concentration is now 0.01 vol%.
- 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.

A



B





A: Structure UbiQ-100. **B:** LC-MS analysis. Mobile phase A = 1% CH_3CN, 0.1% formic acid in water (milliQ) and B = 1% water (milliQ) and 0.1% formic acid in CH_3CN. XBridge BEH300 C18 5µm 4.6x100mm; column T = 40°C, flow= 0.8 mL/min. Gradient: 30–95% over 3.5 min.

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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):

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

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):

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

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_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.).

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

Literature. (1) Tirat et al. *Anal. Biochem.* **2005**, *343*, 244-255. (2) Huang et al. *Methods in Molecular Biology* **2009**, *565*, 127. (3) Levine et al. *Anal. 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.

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