UbiQ
targeting the ubiquitin system

5-TAMRA-Lys(ISG15)-Gly *(mouse sequence, semi-synthetic)*

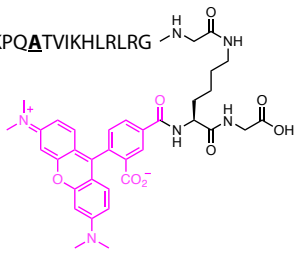
UbiQ code	:	UbiQ-073
Batch #	:	B15112014-001
Amount	:	50 ug, lyophilized powder
Purity	:	≥95% by SDS-PAGE
Mol. Weight	:	18.25 kDa
Storage	:	upon arrival, store powder at −20°C, solution at −80°C. Protect from light and avoid multiple freeze/thaw cycles.

Productsheet

Background. Fluorescence polarization assay reagent for deISGylating enzymes which is based on a 5-carboxytetramethylrhodamine (TAMRA, exc 550 nm, emi 590 nm) modified Lys-Gly sequence that is linked to (mouse) ISG15 (Interferon stimulated gene 15) via a native isopeptide bond with the lysine side-chain.[1-4] ISG15 is a ubiquitin-like protein whose expression is increased following stimulation with type 1 Interferons. A typical substrate concentration is 100 nM. Effective concentrations of deISGylating enzymes depends on specific assay conditions and method of detection. **UbiQ-073** is effectively processed by the deISGylating enzyme USP18 and contains a C78S mutation for higher protein stability.[5]

Sequence

GPGHMAWDLKVKMLGGNDFLVSVTNSMTVSELKKQIAQKIGVPAFQQRLAHQTAVLQDGLTLSSLGLGPSSTVMLVVQN**S**SEPLSILVRNERG

HSNIYEVFLTQTVDTLKKKVSQREQVHEDQFWLSFEGRPMEDKELLGEYGLKPQ**A**TVIKHLRLRG 

* Cys78 mutated to Ser to enhance stability[5]
* Cys144 mutated to Ala during synthesis of the FP reagent
* GPGH sequence is remnant of 3C protease cleavage sequence

sample preparation (100 nM assay concentration)

- dissolve the powder in DMSO (18.25 mg/mL)
- add the DMSO stock to milliQ (please note order) to get a 100 uM stock in milliQ with 10 vol% DMSO.
- the 100 µM stock can then be diluted 1000× in buffer affording a final assay solution of 100 nM UbiQ-073 and a DMSO concentration of 0.01 vol%.
- all stocks are suitable for storage

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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:

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

The confocal optics were adjusted with the average P and S values for TAMRA and the grating factor (G) was determined using a polarization value (L) for 5-TAMRA-Lys-Gly (can be prepared from 100 nM UbiQ-073 by treatment with USP18, e.g. 100 nM) or TAMRA of 50 mP using the following formula:

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

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 equation:

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

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, which were used to determine the Michaelis-Menten constants (K_m, V_{max} and k_{cat}) by fitting the data according to the formula below (where $k_{cat} = V_{max}/[E]$). All experimental data was processed using Ms Excel and Prism 4.03 (GraphPad Software, Inc.).

$$v_i = \frac{V_{max} \times S_0}{K_m + S_0}$$

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) Geurink et al. *ChemBiochem*, **2012**, *13*, 293. (5) Basters et al. *FEBS J.* **2014**, *281*, 1918. *References 4 and 5 are open-access.*