UbiQ

targeting the ubiquitin system

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5-TAMRA-Lys(SUMO-2)-Gly-OH *(human, synthetic)*

UbiQ code : UbiQ-021
Batch # : B29072013-001
Protocol # : P29072013-001

Product Information

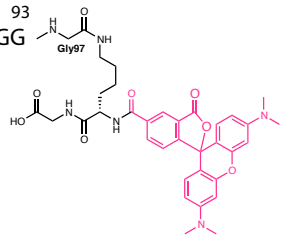
Amount : 100 µg, lyophilized powder
Purity : >95% by SDS-PAGE
Mol. Weight : 13.514 Da by MS
Storage : powder at −20°C, solution at −80°C. Protect from light.
Please avoid multiple freeze/thaw cycles.

Background. Fluorescence polarization assay reagent for deSUMOylating enzymes which is based on a 5-carboxytetramethylrhodamine (TAMRA, exc 550 nm, emi 590 nm) modified Lys-Gly sequence that is linked to (6His tagged) SUMO-2 via a native isopeptide bond with the lysine side-chain.[1-4] Typical substrate concentrations range from 10−100 nM. Effective concentrations of deSUMOylating enzymes can range from 0.01-10 nM but depend on specific assay conditions and method of detection.

Structure 5-TAMRA-Lys(SUMO-2)-Gly-OH

1 10 20 30 40
MGSSHHHHHHSSGLEVLFQGPGS MADEKPKEGV KTENNDHINL KVAGQDGSVV QFKIKRHTPL

50 60 70 80 90 93
SKLMKAYAER QGLSMRQIRF RFDGQPINET DTPAQLEMED EDTIDVFQQQ TGG



* N-terminal 6His tag, protease cleavage site: PreScission™ (LEVLFQ▼GP)

* Cys58 mutated to Ala

Important - sample preparation.

- dissolve the 100 µg **UbiQ-021** (7.4 nmol) in 7.4 µL DMSO (1 mM stock)
- add this DMSO stock to 67 µL milliQ (please note the order of addition), affording a 100 µM solution in milliQ/10vol% DMSO.
- the 100 µM stock can be diluted 1000× in buffer affording a final assay solution with 100 nM **UbiQ-021**. The DMSO concentration is now 0.01 vol%.

Literature. (1) Tirat, A. 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 and El Oualid et al. *ChemBiochem*, **2012**, *13*, 293.

2013
Amsterdam, The Netherlands

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-Lys-Gly (**UbiQ-023**) or TAMRA and the grating factor (G) was determined using a polarization value (L) for 5-TAMRA-Lys-Gly (25 nM, **UbiQ-023**) 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:

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

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.).

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

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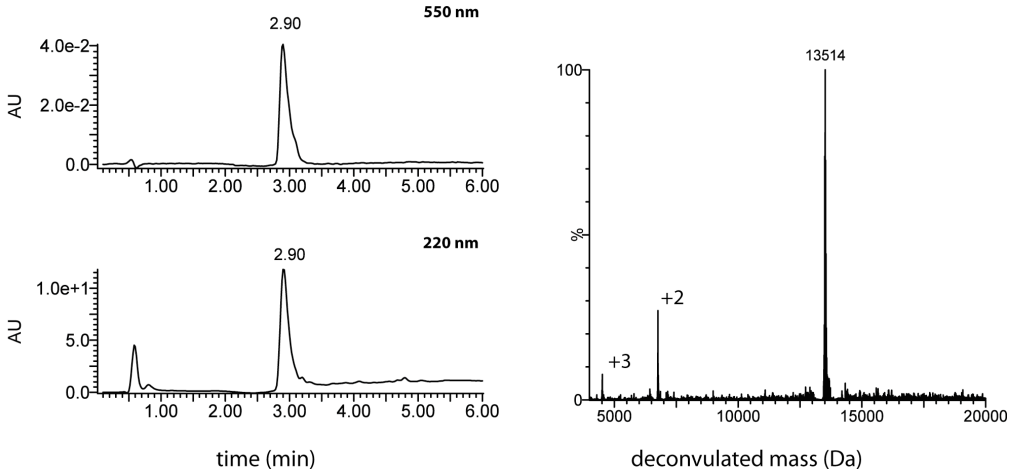
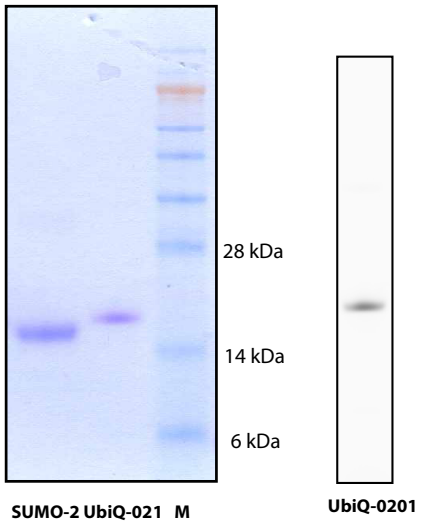


LC-MS analysis. Mobile phase A = 1% CH_3CN, 0.1% formic acid in water (milliQ) and B = 1% water (milliQ) and 0.1% formic acid in CH_3CN. Phenomenex Kinetex C18, (2.1×50 mm), 2.6 µM); flow rate = 0.5 mL/min, runtime = 6 min, column T = 40°C. Gradient: 5% ⇨ 95% B over 3½ min.



SDS-PAGE analysis. 12% SDS-PAGE gel. Marker= SeeBlue® Plus2 (Invitrogen). Left: coommassie stain, right: Fluorescence Scan (Perkin Elmer ProXPRESS 2D, exc 560 emi 590 nM)