

UbiQ

targeting the ubiquitin system

SUMO2 FP (5-TAMRA-Lys(SUMO-1)-Gly-OH, human sequence, semi-synthetic)

UbiQ code : UbiQ-021

Batch # : B29072013-001

Amount : 50 µg, lyophilized powder

Purity : >95%

Mol. Weight : 13.5 kDa

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

Productsheet

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.

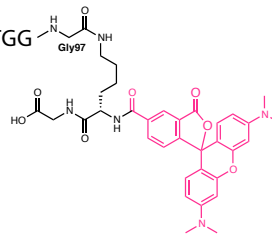
sequence

MGSSHHHHHHSSGLELVFQGPGS MADEKPKEGV KTENNDHINL KVAGQDGSVW QFKIKRHTPL

SKLMKAY**A**ER QGLSMRQIRF RFDGQPINET DTPAQLEMED EDTIDVFQQQ TGG

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

* Cys58 mutated to Ala



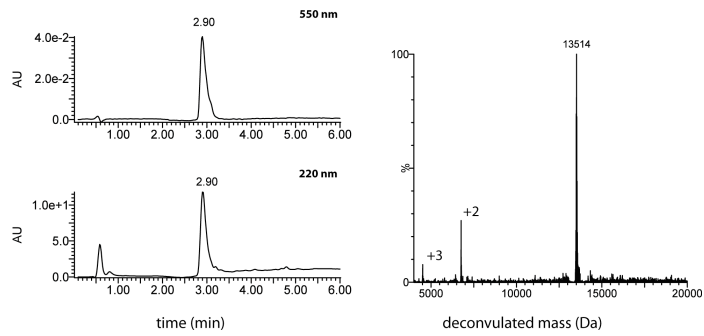
sample preparation

- dissolve the powder in DMSO to a final concentration of for example 1000 µM (25 ug in 1.9 uL DMSO)
- add this DMSO stock to the required buffer to a final concentration of 100 nM (final DMSO concentration is now 0.01 vol%).
- one can also prepare a stock in milliQ of 100 uM by diluting the DMSO stock 10x in milliQ; the DMSO conc is now 10 vol%.
- Typical concentrations for assays: 25–100 nM. See reference 4 for full experimental details.

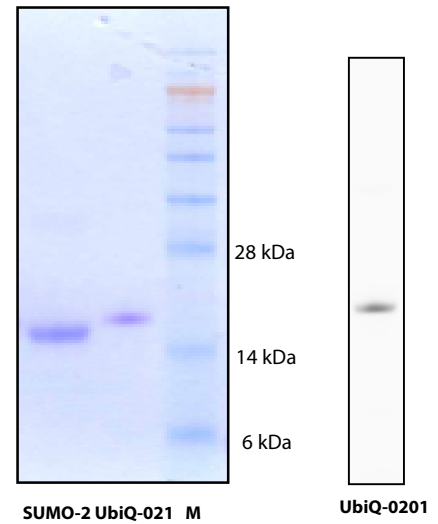
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A



B



A: LC-MS analysis. Mobile phase A = 1% CH₃CN, 0.1% formic acid in water (milliQ) and B = 1% water (milliQ) and 0.1% formic acid in CH₃CN. 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. **B: SDS-PAGE analysis.** 12% SDS-PAGE gel. Marker= SeeBlue® Plus2 (Invitrogen). Left: coomassie stain, right: Fluorescence Scan (Perkin Elmer ProXPRESS 2D, exc 560 emi 590 nM)

General protocol fluorescence polarization assay

FP assays were performed on a PerkinElmer Wallac EnVision 2100 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 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):

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

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 μ 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.).

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

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