UbiQ
targeting the ubiquitin system

SUMO2 FP (5-TAMRA-Lys(SUMO-2)-Gly, *human sequence, C58A, semi-synthetic)*

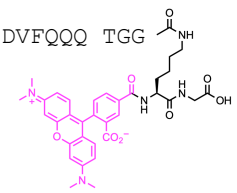
UbiQ code	:	UbiQ-021
Batch #	:	B29072013-001
Amount	:	50 ug, 1 mM in DMSO (3.7 µL)
Purity	:	>95%
Mol. Weight	:	13.5 kDa
Storage	:	upon arrival, powder at −20°C, solution at −80°C. Protect from light and avoid multiple freeze/thaw cycles.

Productsheet

Background. UbiQ-021 is fluorescence polarization assay reagent for deSUMOylating enzymes. It 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. 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

MGSSHHHHHHSSGLEVLFQGPGS MADEKPKEGV KTENNDHINL KVAGQDGSVV QFKIKRHTPL
SKLMKAYAER QGLSMRQIRF RFDGQPINET DTPAQLEMED EDTIDVFQQQ TGG



* N-terminal 6His tag, protease cleavage site: PreScission™ (LEVLFQ▼GP)
* Cys58 mutated to Ala

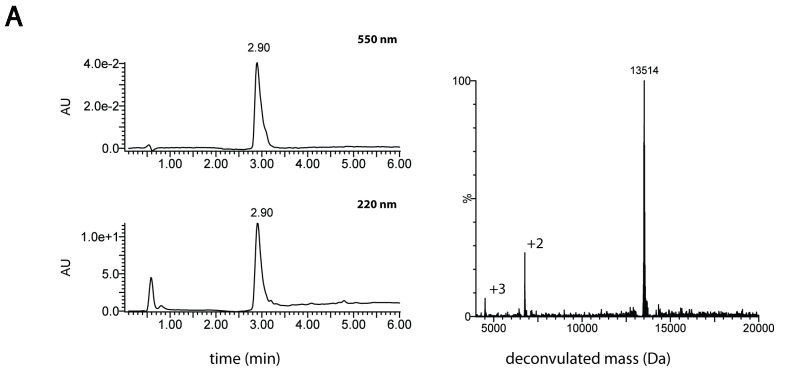


Figure 1. A: LC-MS analysis. Mobile phase A= 1% CH_3CN, 0.1% formic acid in water (milliQ) and B= 1% water (milliQ) and 0.1% formic acid in CH_3CN. Phenomenex Kinetex C18, (2.1×50 mm), 2.6 µM); flow rate = 0.5 mL/min, column T = 40°C. Gradient: 5-95%B over 3½ min. B: SDS-PAGE analysis (12% SDS-PAGE gel), lane 1= SUMO2, lane 2= UbiQ-021, M= SeeBlue® Plus2 (Invitrogen). CBB staining. C: Fluorescence Scan (Perkin Elmer ProXPRESS 2D, exc 560 emi 590 nM) UbiQ-021.

sample preparation

- Example: prepare a stock in milliQ of 100 uM by diluting the DMSO stock 10× in milliQ; the DMSO conc is now 10 vol%.
- Add the aq. stock to your assay buffer to a final concentration of 100 nM (DMSO concentration is now 0.01 vol%).
- Typical concentrations for assays: 25−100 nM. See reference 5 for full experimental details.

Literature. (1) Tirat et al. *Analyt Biochem* **2005**, *343*, 244. (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.

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General protocol fluorescence polarization assay

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):

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

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):

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

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.).

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