UbiQ
targeting the ubiquitin system

hISG15 FP *(human sequence, semi-synthetic)*

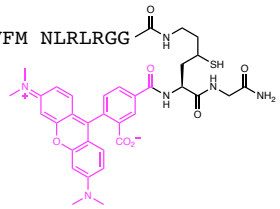
UbiQ code	:	UbiQ-287
Batch #	:	B01102020-001
Amount	:	62.5 uL, 0.4 mg/mL (22 uM) in 50 mM HEPES pH 7.5, 150 mM NaCl, 2 mM DTT= 25 ug
Purity	:	≥95% by SDS-PAGE
Mol. Weight	:	18.21 kDa
Storage	:	upon arrival, store solution at –20°C, protected form light. As human ISG15 proteins tend to precipitate (during freeze/thaw cycles), please avoid long-term storage (at –80°C) and multiple freeze/thaw cycles.

Productsheet

Background. UbiQ-287 is a fluorescence polarization assay reagent for deISGylating enzymes. It is based on a 5-carboxytetramethylrhodamine (TAMRA, exc 550 nm, emi 590 nm) modified ^{thio}Lys-Gly sequence (see sequence), linked via an isopeptide bond to human ISG15 (Interferon stimulated gene 15).

sequence

GPLGS MGWDLTVKML AGNEFQVSLS SSMSVSELKA QITQKIGVHA FQQRLAVHPS GVALQDRVPL ASQGLGPGST VLLVVDKCDE PLNILVRNNK GRSSTYEVRL TQTVAHLKQQ VSGLEGVQDD LFWLTFEGKP LEDQLPLGEY GLKPLSTVFM NLRLRGG



* GPLGS sequence is a remnant of 3C protease cleavage sequence

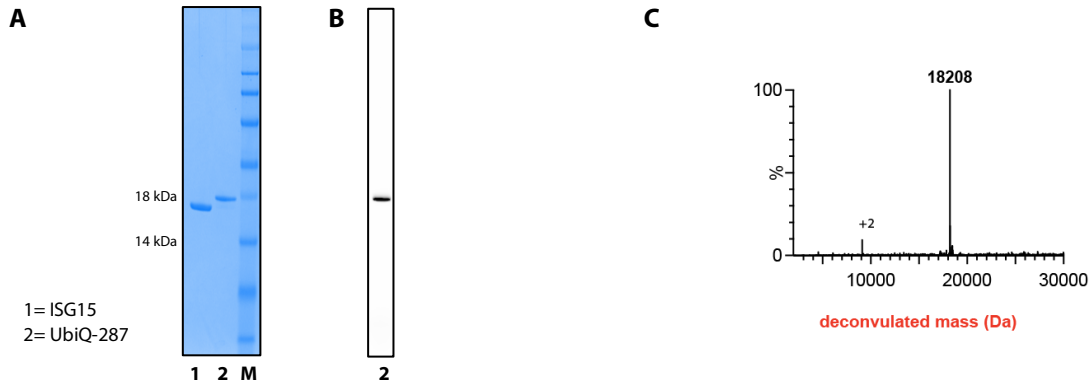


Figure 1. A: SDS-PAGE analysis, Marker= SeeBlue® Plus2 (Invitrogen), 12% Bolt Bis-Tris Plus gel (Lifetechnologies) in MES buffer at 190V, staining with *Instant Blue*. B: fluorescent scanning. C: MS analysis.

For assay details please see page 2 and open-access references 1 and 2.

1) Geurink et al. *ChemBiochem*, **2012**, *13*, 293: https://chemistry-europe.onlinelibrary.wiley.com/doi/full/10.1002/cbic.201100706

2) Basters et al. *FEBS J.* **2014**, *281*, 1918: https://febs.onlinelibrary.wiley.com/doi/10.1111/febs.12754

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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 (I):

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

The confocal optics were adjusted with the average P and S values for 5-TAMRA and the grating factor (G) was determined using a polarization value (L) for 5-TAMRA-^{thio}Lys-Gly (can be prepared from UbiQ-287 by treatment with high concentration of USP18, e.g. 100 nM) or 5-TAMRA of 50 mP using the following formula (II):

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

The assays were performed in "non binding surface flat bottom low flange" black 384-well plates (Corning) at room temperature in buffer containing 1 mg/mL CHAPS (*3-[(3-cholamidopropyl) dimethylammonio] propanesulfonic acid*) and 0.5 mg/mL BGG (*bovine gamma globulin*). 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 (III):

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

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 (IV) below (where $k_{cat} = V_{max}/[E]$).

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