

UbiQ

targeting the ubiquitin system

Ac-ISG15^{prox}-Rh110MP (mouse sequence, proximal= C-terminal domain, synthetic)

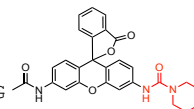
UbiQ code : UbiQ-127
Batch # : B01055021-001
Amount : 50 ug, lyophilized powder
Purity : $\geq 95\%$
Mol. Weight : 9.69 kDa
Storage : upon arrival, powder at -20°C , solution at -80°C . Please store dark and avoid multiple freeze/thaw cycles.

Productsheet

Background. Ac-ISG15^{prox}-Rh110MP is a quenched, fluorescent substrate for ISG15 proteases based on the C-terminal domain of mouse ISG15. Cleavage of the amide bond between the C-terminal Gly and rhodamine110 (Rh110) moiety releases the highly fluorescent Rh110-morpholinecarbonyl (Rh110MP). Overall, UbiQ-127 offers the excellent properties of a quenched Ubi-Rh110X substrate with a very high fluorescence intensity after proteolytic cleavage.

A

Ac-SEPLSILVRNERGHNSNIYEVFLTQTVDTLKKKVSQREQVHEDQFWLSFEGRPMEDEKELLGEYGLKQPSTVIKHLRLRGG



B

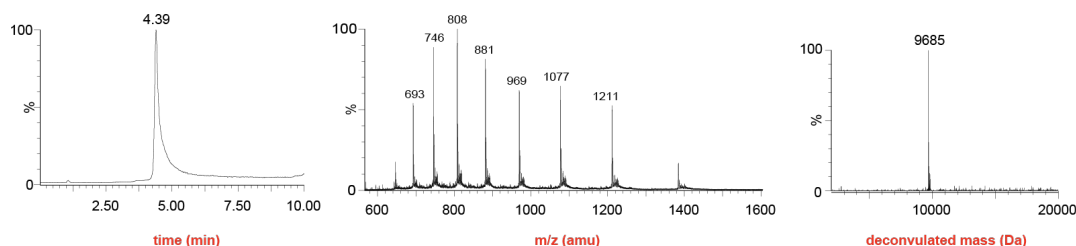


Figure 1. A: sequence. B: LC-MS analysis. Mobile phase A= 1% CH₃CN, 0.1% formic acid in water and B= 1% water and 0.1% formic acid in CH₃CN. XBridge BEH300 C18 3.5 μm 4.6x100mm; column T= 40°C, flow= 0.8 mL/min. Gradient: 20–50%B over 6.5 min.

important: sample preparation

- dissolve the powder in DMSO
- DMSO stocks may range (for example) from 1 mg/mL (103 μM) to 40 mg/mL (4.12 mM)
- add the DMSO stock to milliQ and mix
- *to ensure proper folding, we advise to buffer the aqueous DMSO stock first to 50 mM sodium acetate pH 4.5*
- next, buffer as desired. For example:
 - 1) dissolve 50 ug UbiQ-127 in 2 uL DMSO and add to 91 uL milliQ
 - 3) add 2 uL of a 2.5M sodium acetate pH 4.5 stock – this UbiQ-127 stock ($\pm 54 \mu\text{M}$) can be diluted directly into assay buffer when working with, for example, an assay concentration of 1 μM or lower.
 - 4) for assays where higher concentrations of UbiQ-127 are required, the sodium acetate stock can be buffered with assay buffer; for example by adding 5 uL of 1M HEPES pH 7.5 – final conc.= 0.5 mg/mL= 51 μM (2 vol% DMSO)

recommended filter settings for Rh110MP

- $\lambda_{\text{ex}} = 492 \text{ nm}$, $\lambda_{\text{emi}} = 525 \text{ nm}$
- bandwidth: $\pm 8 \text{ nm}$

Literature. (1) Basters et al. *Nat Struct Mol Biol* **2017**, *24*, 270. (2) Terentyeva et al. *Biocon Chem* **2011**, *22*, 1932. (3) Hassiepen et al. *Analyt Biochem* **2007**, *371*, 201.

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