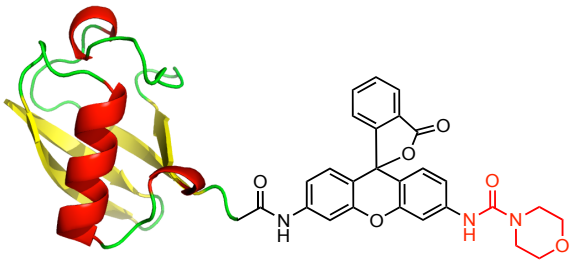
UbiQ
targeting the ubiquitin system



Ub-Rh110MP *(human sequence, synthetic)*

UbiQ code	:	UbiQ-126
Batch #	:	B01092015-001
Amount	:	50 ug, lyophilized powder
Purity	:	≥95%
Mol. Weight	:	8.97 kDa
Storage	:	upon arrival powder at –20°C, solution at –80°C. Please avoid multiple freeze/thaw cycles.

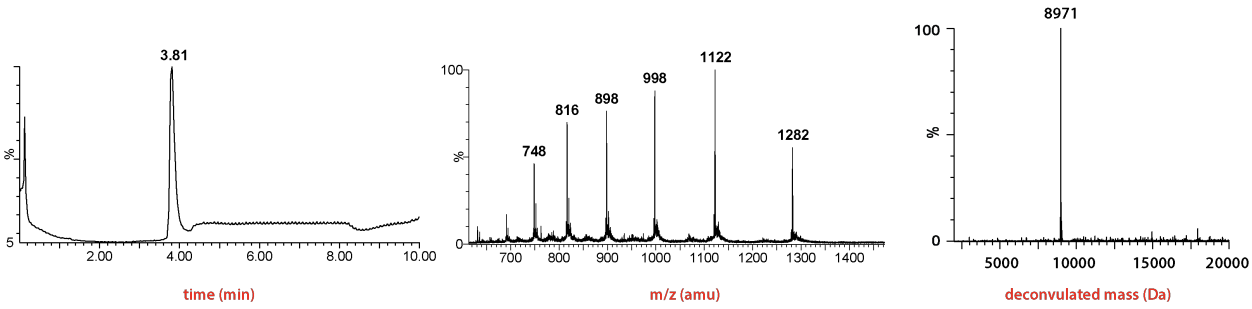
Productsheet

Background. Ub-Rh110MP is a new type of quenched, fluorescent substrate for deubiquitinating enzymes (DUBs). Cleavage of the amide bond between Ub Gly76 and the Rhodamine110 moiety releases the highly fluorescent Rh110-morpholinecarbonyl (Rh110MP, exc/emi= 492/525 nm).[1,2] Rh110MP exhibits a higher fluorescence intensity than the classical Rh110Gly fluorophore of Ub-Rh110Gly (UbiQ-002).[3] UbiQ-126 is prepared by total chemical synthesis.[4]

- **keep the excellent properties of the classic ubiquitin-Rh110 substrate (Figures 1 - 3)**
- ***with* increased fluorescence intensity after proteolytic cleavage (Figures 1C and 3).**

sequence

MQIFVKTLTGKTITLEVEPSDTIENVKAKIQDKEGIPPDQQRLIFAGKQLEDGRTLSDYNIQKESTLHLVLRLRGG-**Rh110MP**



LC-MS analysis. Mobile phase A= 1% CH_3CN, 0.1% formic acid in milliQ and B= 1% milliQ and 0.1% formic acid in CH_3CN. XBridge BEH300 C18 5µm 4.6x100mm; column T= 40°C, flow= 0.8 mL/min. Gradient: 30–60% over 6.5 min.

important: sample preparation

- dissolve the powder in DMSO: DMSO stocks can range from 0.9 mg/mL (100 uM) to 40 mg/mL (4.45 mM)
- add the DMSO stock to milliQ (please note the order of addition) and mix
- buffer the aq. solution as desired (using 1M HEPES or 1M Tris for example)
- a final assay stock of 100 nM will contain 0.1 vol% DMSO when prepared from a 100 uM DMSO stock

Literature. (1) Lavis et al. *ACS Chem Biol* **2006**, *1*, 252. (2) Terentyeva et al. *Bioconj Chem* **2011**, *22*, 1932. (3) Hassiepen et al. *Analytical Biochem* **2007**, *371,* 201. (4) El Oualid et al. *Angew Chem Int Ed* **2010**, *49*, 10149.

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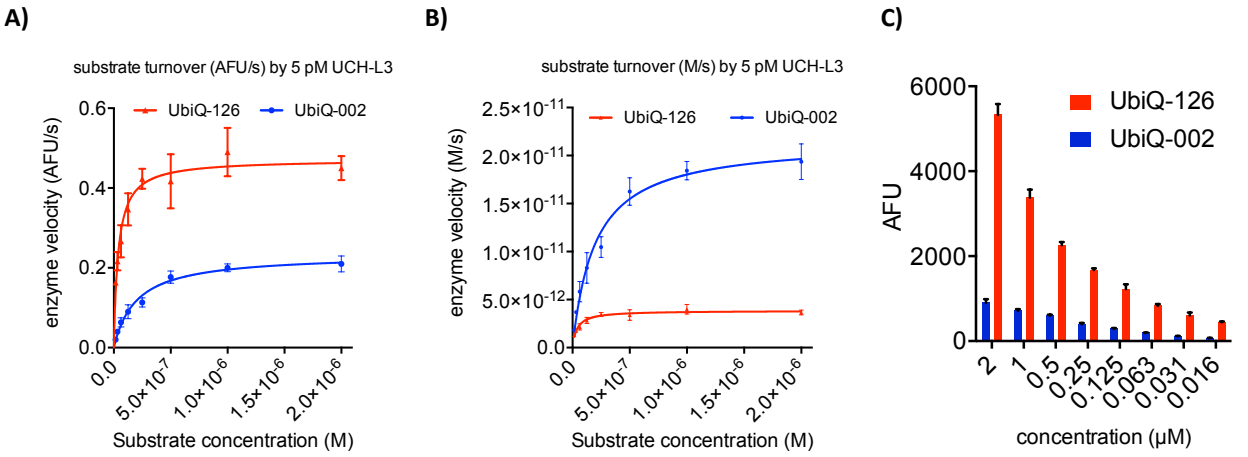

Figure 1. Michaelis-Menten kinetics of UbiQ-126 (Ub-Rh110MP) and UbiQ-002 (Ub-Rh110Gly), turned over by 5 pM UCH-L3. Enzyme kinetics were determined in 384 well format (30 uL per well) on a *BMG Clariostar plate reader* measuring fluorescence intensity at λ_{exc} 487 ± 14 nm; λ_{emi} 535 ± 30 nm; 40 flashes per well.

A) enzyme velocity represented as AFU/s versus substrate concentration. AFU: arbitrary fluorescence units.

B) enzyme velocity represented as M/s versus substrate concentration.

C) fluorescence intensities determined at 30 min turnover by 5 pM UCH-L3, error bars are SD (n=3).

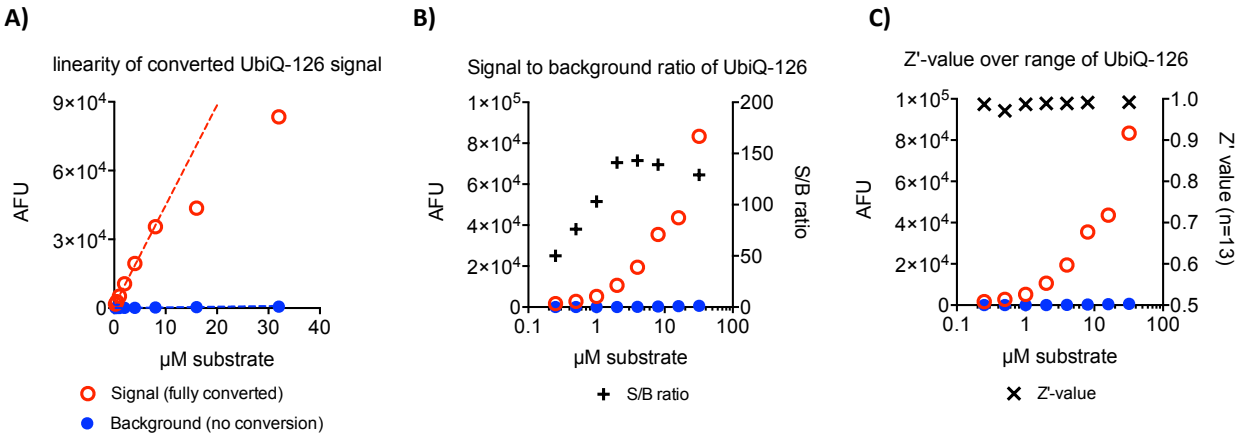

Figure 2. Fluorescence signal versus background of UbiQ-126 (Ub-Rh110MP). Fluorescence intensities were measured of various concentrations of **UbiQ-126** (background) and fully converted **UbiQ-126** by 1 µM USP7 (signal).

A) the fluorescence signal of processed **UbiQ-126** is linear up to 8 µM.

B) signal-to-background ratios over a concentation range of **UbiQ-126**.

C) Z'-values over a concentation range of **UbiQ-126**, determined over 13 replicates. Fluorescence intensities were measured in 384 well format on a *BMG Pherastar plate reader* at λ_{exc} 485 ± 16 nm; λ_{emi} 520 ± 10 nm.

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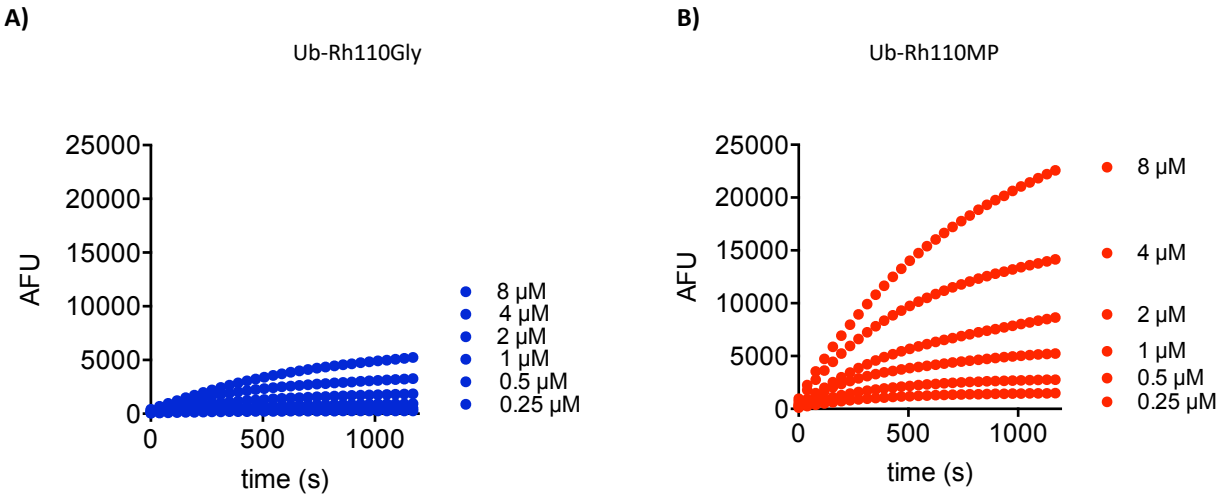

Figure 3. Progress curves of the conversion of UbiQ-002 (Ub-Rh110Gly) and UbiQ-126 (Ub-Rh110MP) by 1 nM USP7. Fluorescence intensities were measured in 384 well format on a *BMG Pherastar plate reader* at λ_{exc} 485 ± 16 nm; λ_{emi} 520 ± 10 nm. AFU: arbitrary fluorescence units.
A) conversion of **Ub-Rh110Gly** (blue dots) at the indicated concentrations.
B) conversion of **Ub-Rh110MP** (red dots) at the indicated concentrations.