

Mca-KKVAPYPME-Dap(Dnp)-NH₂

UbiQ code : UbiQ-141

Batch # : B01052016-001

Aliquote size : 1.00 mg, lyophilized powder

Purity : ≥95% by RP-HPLC

Mol. Weight : 1.53 kDa

Storage : powder and DMSO solution at -20°C. Please store in dark environment and avoid multiple freeze/thaw cycles.

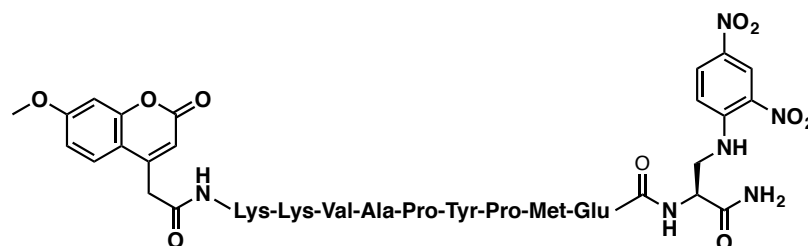
Productsheet

Background. UbiQ-141 is a long fluorogenic reagent for monitoring ATP dependant protease activity (like in the 26S proteasome and ClpP protease) and is based on the LF-2 peptide.¹⁻⁴

Key characteristics.

- **UbiQ-141 is only processed by activated proteasomes (Figure 1),¹** in contrast to the commonly used tetrapeptide based proteasome activity assay reagents (such as Suc-LLVY-AMC).
- **UbiQ-141 exhibits a much more pronounced stimulation of proteasome activity than tetrapeptide based proteasome activity assay reagents.** This was shown to be the case when *M. tuberculosis* 20S core particles were activated with the cofactor Rv3780 and activity was analysed using UbiQ-141 and Suc-LLVY-AMC.¹
- **UbiQ-141 is processed by human and bacterial proteasomes.**

Sequence.



peptide reconstitution

- dissolve 100 µg of UbiQ-141 in 65 µl DMSO to give a 1 mM stock solution (50×)
- this can be in aliquoted and stored at -20°C.
- before an experiment, an aliquot is thawed and diluted 10× in peptide degradation assay reaction buffer (50 mM Tris pH 8, 5 mM MgCl₂) to make a 5× working dilution.

peptide degradation assay

- 1 µg freshly purified *M. tuberculosis* His6-tagged 20S core particle is mixed with 3.44 µg purified His6-tagged PafE in peptide degradation assay reaction buffer (50 mM Tris pH 8, 5 mM MgCl₂) in a total volume of 240 µl.
- mixtures are incubated at 37°C for 30 min to promote complex formation, and then incubated at room temperature for 10 minutes to cool.
- triplicate 60 µl aliquots are transferred to a 96 well plate, and 15 µl of peptide substrate (5×) is added to each well to give a final peptide concentration of 20 µM.
- degradation rate was assessed by monitoring fluorescence generated over time at room temperature (excitation: 340 nm; emission: 405 nm).

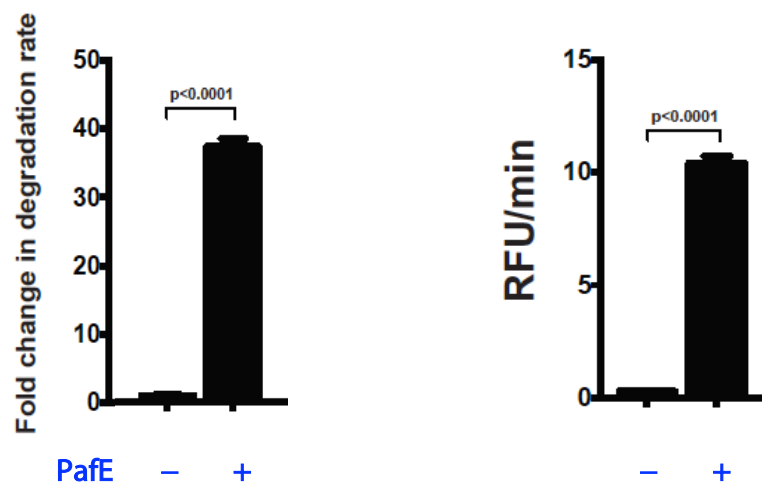


Figure 1. Degradation rate of UbiQ-141 by *M. tuberculosis* 20S proteasom, without or with a 10-fold molar excess of PafE (courtesy of Jordan Jastrab and Dr Heran Darwin, NYU School of Medicine, USA).

Literature. (1) Jastrab et al. *Proc. Natl. Acad. Sci. USA* **2015**, *127*, E1763. (2) Bai et al. *Proc. Natl. Acad. Sci. USA* **2015**, *127*, E1983 (3) Smith et al. *Mol. Cell* **2007**, *27*, 731. (4) Smith et al. *Mol. Cell* **2005**, *20*, 687.