UbiQ
targeting the ubiquitin system

Mca-KKVAPYPME-Dap(Dnp)–NH_2 *(synthetic)*
UbiQ code : UbiQ-141
Batch # : B01052016-001
Amount : 1.00 mg, lyophilized powder
Purity : ≥95%
Mol. Weight : 1.53 kDa
Storage : upon arrival, store at –20°C. Please store dark and avoid multiple freeze/thaw cycles.

Productsheet

Background. UbiQ-141 (Figure 1A) 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.

- UbiQ-141 is only processed by activated proteasomes (Figure 2), 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.
- UbiQ-141 is processed by human and bacterial proteasomes.

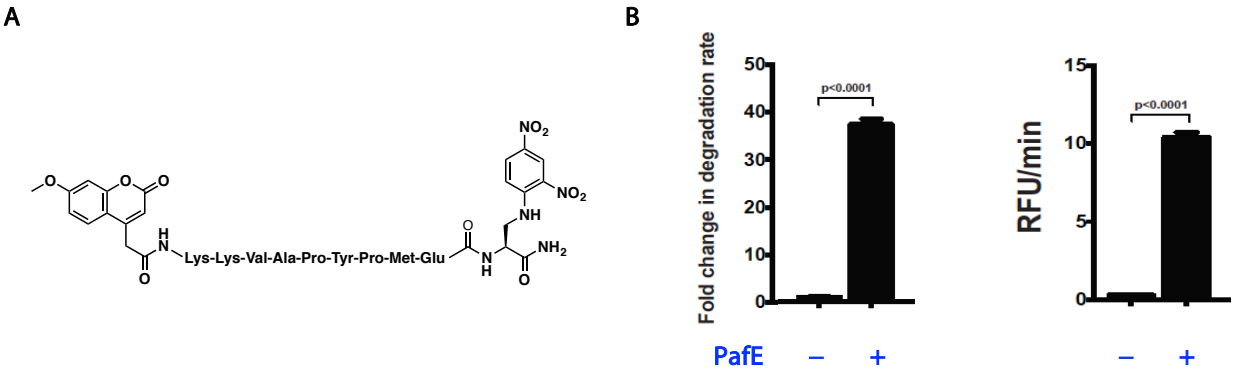

Figure 1. A: structure. B: degradation rate of UbiQ-141 by *M. tuberculosis* 20S proteasoom, without or with a 10-fold molar excess of PafE (*courtesy of Jordan Jastrab and Dr Heran Darwin, NYU School of Medicine, USA*).

peptide reconstitution and general assay set-up

- 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 pH8, 5 mM $MgCl_2$) to make a 5× working dilution.
- 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 buffer (50 mM Tris pH 8, 5 mM $MgCl_2$) 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
- $\lambda_{excitation}$= 340 nm, $\lambda_{emission}$= 405 nm

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.

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