UbiQ
targeting the ubiquitin system

peptide degradation assay

version	V1, Sept-2016
applicable for	UbiQ Proteasome assay reagent Mca-KKVAPYPME-Dap(Dnp)–NH2
reference	Jastrab, J. B., et al. An adenosine triphosphate-independent proteasome activator contributes to the virulence of Mycobacterium tuberculosis. *Proc Natl Acad Sci USA*. **14**, E1763-E1772 (112) doi: 10.1073/pnas.1423319112

protocol

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 buffer (50 mM Tris pH 8, 5 mM MgCl2) in a total volume of 240µl.
- 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).

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