



bioluminescence assay

version	V1, 17-9-2015
applicable for	UbiQ assay reagents
reference	Orcutt, S.J., et al. Bioluminescence Assay Platform for Selective and Sensitive Detection of Ub/Ubl Proteases. <i>Biochim. Biophys. Acta.</i> 1823 , 2079-2086 (2012).

protocol

1. Dilute 250 nM assay reagent (Ub-AMC/Ub-Rho110Gly/Ub-Luc) into reaction buffer containing 50 nM Tris, pH 8.0, 0.15 M NaCl, 5 nM DTT, and 0.05% CHAPS.
2. Assemble the reactions in 96-well black plates and initiate the reaction by the addition of serially diluted DUBs of interest. Monitor the fluorescence intensity immediately after DUB addition (30 min at intervals of 30s) on an EnVision Multi-Label Plate Reader (Perkin Elmer) using the excitation/emission filter pair specifically determined for the reagent (340/460 nm for Ub-AMC; 485/535 nm for Ub-Rho110Gly; 400/505 nm for Ub-Luc).