The process of HDAC11 Assay Development: a kit-free method

The Enzo's Fluor-de-Lys-Green kit when used to monitor the activity of HDAC11 in a preliminary test showed very less activity for the protein (only ~two times of fluorescence signal for a reaction with 17.5 µM HDAC11 for 90 min, Fig 3, *data set*). It might be possible that the substrate used in the kit is not an appropriate/preferred substrate for HDAC11. In order to overcome this, a principally identical reaction set-up was designed separately from the kit, wherein Boc-Lys-(TFA)-AMC (Bachem I-1985.0050) was used as the substrate (Madsen and Olsen, 2012). The reaction recipe is described in Table 1. From here, the total reaction volumes are further reduced to 15 µl (7.5 µl reaction+ 7.5 µl developer), from which 10 µl is transferred to the 384-well black Grenier plate for measurement. The Fluor DE LYS developer II concentrate (BML-KI176) was purchased from Enzo as a 5X stock. Trichostatin A (TSA), a HDAC inhibitor was also included in the developer solution (Its reported IC_{50} for HDAC11 with another substrate was in low nM range (*ref.*). Accordingly with this different substrate, a conc. of 20 µM was used in the final mix upon suggestion by a collaboration partner). For AMC as the fluorophore, the excitation and emission filters used with the Synergy4 plate reader were 340/30 nm and 460/40 nm, respectively. As performed previously in line with the instructions from the Enzo manual which mentions that the fluorescence signal stabilizes after 20-30 min upon the addition of the developer, the reaction was quenched with the developer at the desired time points and the measurements were taken together in the end for all the time intervals following a 30 min incubation of the 60 min time point with the developer.

Table 1. Reaction set-up to monitor the activity of recombinant HDAC11.

7.5 µl Reaction volume	
HDAC11 (µM)	0.05
Boc-Lys-(TFA)-AMC (µM)	50
Assay buffer	20 mM HEPES, pH 8.0, 137 mM NaCl, 2.7 mM KCl, 1 mM MgCl2 0.5 mg/ml BSA (added freshly)
Reaction time at RT (25 ºC)	0-60 min
7.5 µl Developer	
Developer conc. (5X stock)	1X
TSA (Trichostatin A)	40 µM
Incubation time (with respect to last time point)	30 min

As per Fig 1, HDAC11 appears to be more active (~10 times fluorescence at 60 min) with this new substrate (Boc-Lys-(TFA)-AMC) as compared to that used in the kit (~2 times fluorescence at 90 min, Fig 3, *data set).* As a note, no blank (sample without protein, i.e., only substrate) was used in this preliminary experiment (performed in singlet).

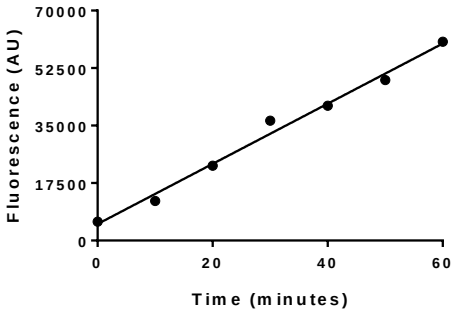

Fig 1. Deacetylation of Boc-Lys-(TFA)-AMC by HDAC11.

References:

1. Madsen, A.S., and Olsen, C.A. (2012). Profiling of substrates for zinc-dependent lysine deacylase enzymes: HDAC3 exhibits decrotonylase activity in vitro. Angewandte Chemie *51*, 9083-9087.