The process of HDAC11 Assay Development: enzyme stability

Before calculating the Km for HDAC11 by performing a series of time-course experiments, it is important to know the duration of the stability of the protein at the temperature being used for the assays. Here, HDAC11 assays are performed at room temperature. Table 1 describes the reaction recipe.

Table 1. Reaction recipe for enzyme stability.

7.5 µl Reaction volume	
HDAC11 (µM)	0.125
Boc-Lys-(TFA)-AMC (µM)	200
Assay buffer	20 mM Bicine, pH 8.5, 137 mM NaCl, 2.7 mM KCl, 1 mM MgCl2 0.5% BSA (added freshly)
Reaction time at RT (25 ºC)	30 min
7.5 µl Developer	
Developer conc. (5X stock)	0.1 X
Incubation time	1 hour

The stock of HDAC11 was diluted to 0.125 µM and left at RT to mimic the conditions of the experimental set-up that would be used later to study the time-course kinetics. This enzyme was used for activity measurements over a period of 5 hours. At each time point, the reaction was measured for period of 30 min. The results in Fig 1 are depicted as Fold change in Fluorescence (activity of every time point normalized against that of 0 min). The results show that the enzyme is active at RT for atleast 5 hours, the time duration being tested here.

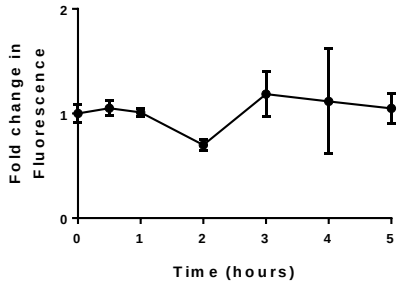

Fig 1. The stability of HDAC11 at RT over a period of 5 hours.