The process of HDAC11 Assay Development: time-course study follow-up

So far for all the reactions, 1X of the developer concentration was being used (stock as supplied by Enzo is 5X). Since this concentration is high (considering the screening of large libraries and thus, the cost of the resource that will be used up), optimizing the developer concentration is an important parameter.

Table 1 describes the recipe for the reaction for the optimization of developer concentration. Since 2 µM of HDAC11 gave high signals previously (Fig 1, *dataset*), it was chosen for the assay.

Table 1. Reaction recipe for optimizing the developer concentration.

7.5 µl Reaction volume	
HDAC11 (µM)	2
Boc-Lys-(TFA)-AMC (µM)	200
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 min
7.5 µl Developer	
Developer conc. (5X stock)	varies
TSA (Trichostatin A)	40 µM
Incubation time	1 hour

Fig 1 shows that lowering the developer concentration from 1X to 0.1X, does not hinder the signal development.

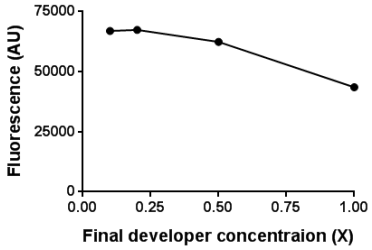

Fig 1. Effect of reducing the developer concentration on the fluorescence signals.

Henceforth, a lower developer concentration of 0.1X was chosen to be used from further on.

From the prior time-course data, it appeared that HDAC11 exhibits pre-steady state kinetics. At higher concentrations of HDAC11 (>0.5 µM), a high fluorescence at reaction time of 0 min was observed, following which the signals did not change. As mentioned in the inferences section of that dataset, it could be because all the substrate was used up at time 0 min (Fig 1, *dataset*).

The signals for these HDAC11 reactions are read post addition of the developer, which as mentioned earlier (at the start of the assay development), was based on the recommendations of the Enzo kit manual, wherein the signals stabilize after 10-15 min and thus, the samples could be read at any time after that (Fig 2). Accordingly, in my time-course study, the developer added at each time point was left for

incubation, and all the samples were read together after 30 min of developer incubation for the last time point. Henceforth, all the time point samples in the time-course experiment were incubated with the developer for varying times.

5. Allow HDAC reactions to proceed for desired length of time and then stop them by addition of *Fluor de Lys*® Developer (50 µl). Incubate plate at room temperature (25°C) for 10-15 min. or transfer to fluorometer to monitor signal development Once fully developed, the signal is stable and can even be read hours later.

Fig 2. Excerpt from Enzo manual (BML-AK530 Instruction Manual).

In order to question the effect of this varying time of developer incubation on the fluorescence signals, a reaction set-up described in Table 2 was made, wherein the time of developer incubation was varied for a 30 min HDAC11 reaction. Since 0.125 µM of HDAC11 gave reasonably detectable signals (Fig 1, *dataset*), this concentration was used for optimization purposes.

Table 2. Reaction recipe for the study of time of incubation with the developer.

7.5 µl Reaction volume	
HDAC11 (µM)	0.125
Boc-Lys-(TFA)-AMC (µM)	200
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)	30 min
7.5 µl Developer	
Developer conc. (5X stock)	0.1X
TSA (Trichostatin A)	40 µM
Incubation time	varies

Fig 3 shows that the longer the developer is incubated, the more is the signal development and that the signals are not stabilized in 10-15 min, as mentioned in the Enzo manual. Henceforth, in the 90/120 min time-course study, since the earlier time points were incubated for a longer time as compared to the following later time-points, the measurement of signals was not accurate.

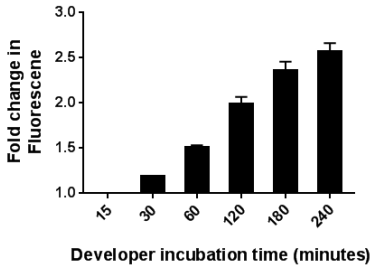

Fig 3. Effect of varying the incubation time with developer on the fluorescence signals.