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

Once the developer optimization trials were made (*dataset*), it was clear that the discrepancies in the data were due to varying developer incubation times (Fig 1, *dataset*). For further optimizations, preliminary tests were made to check the effect of some additives on the activity of HDAC11 over a 30 min reaction period. As per the previous data obtained (*dataset*), the activity (change in fluorescence signals) observed over a 1.5-2 hour period was observed to be very low. Thus, this preliminary test was performed to monitor the drastic increase in the activity upon the addition of a specific additive, if any.

Table 1 describes the reaction recipe to check the effect of additives.

Table 1. Reaction recipe for optimizing the developer concentration.

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% BSA (added freshly)
Reaction time at RT (25 ºC)	0, 30 min
7.5 µl Developer	
Developer conc. (5X stock)	0.1 X
Incubation time	1 hour

Fig 1 shows the signals in the form of fold change in fluorescence measured at 30 min with respect to 0 min (30 min signals normalized to that of 0 min, 0 min readings treated as 1 upon normalization for fold change). Blank (sample with no protein and no additive for this experiment) was measured for each time point and subtracted individually.

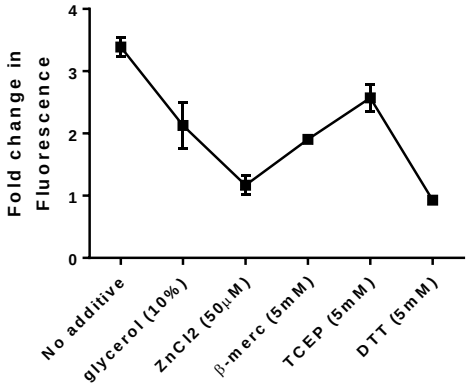

Fig 1. Effect of additives on the activity of HDAC11 represented as fold change at 30 min (against that of 0 min, which was considered as 1).

Observations:

1. Addition of ZnCl2 rendered the protein inactive.
2. Addition of DTT also rendered the protein inactive.
3. Maximum activity was seen without the addition of any additive, followed upon by TCEP, glycerol and β-mercaptoethanol (β-merc).

Inferences:

1. The concentration of protein used is 0.125 µM and that of ZnCl2 used is 50 µM (400 times that of the protein). Such a high concentration could be detrimental (causing precipitation) of the protein.
2. The only one concentration of the additive being used here for the preliminary test might not be able to show the effect which might exist (at a comparatively higher or lower concentration) and thus, becomes clear if an appropriate titration of each additive is performed.

Since the maximum difference in the fold change between 30 min and 0 min was observed in the case of No additives, further optimizations will be performed without the addition of any additive in the buffer, for now.

Although it should be noted, the blank used in this preliminary test for every sample contained no protein-no additive. A detailed screen for additives should use no protein-additive as a blank corresponding to every additive.

Also, the fold change observed here in case of No additives sample (~3 times change) should not be compared and confused with the previous data (~1.5 times change) for 60 min (Fig 3, *dataset*). The aim of the experiments is different and the signals plotted in the previous data were normalized against the reading for 15 min of incubation time.