The process of HDAC11 Assay Development: buffer screening

From the previous preliminary data, it was observed that HDAC11 assay works best with no addition of additives, as of now (Fig 1, *dataset*). In order to check the best buffer conditions for HDAC11 activity, a buffer screen was performed.

(from vector after tag cleavage)
GSMLHTTQLYQHVPETRWPIVYSPRYNITFMGLEKLHPFDAGKWGKVINFLKEEKLLSDSMLVEAREASEE
DLLVVHTRRYLNELKWSFAVATITEIPPVIFLPNFLVQRKVLRPLRTQTGGTIMAGKLAVERGWAINVGGG
FHHCSSDRGGGFCAYADITLAIKFLFERVEGISRATIIDLDAHQGNGHERDFMDDKRVYIMDVYNRHIYPGD
RFAKQAIRRKVELEWGTEDDEYLDKVERNIKKSLQEHLPDVVVYNAGTDILEGDRLGGLSISPAGIVKRDE
LVFRMVRGRRVPILMVTSGGYQKRTARIIADSILNLFGLGLIGPESPSVSAQNSDTPLLPPAVP

Fig 1. HDAC11 protein sequence.

The pI of the protein is 7.3 (protein sequence described in Fig 1, theoretical pI as calculated via *ExPASy ProtParam* online tool) and thus, the buffers being tested were in the range of pH 8-9 (to avoid the pH near its isoelectric point that will cause it to precipitate). Table 1 describes the recipe for the reaction set-up.

Table 1. Reaction recipe for buffer screening.

7.5 µl Reaction volume	
HDAC11 (µM)	0.125
Boc-Lys-(TFA)-AMC (µM)	200
Assay buffer	20 mM buffer, pH 8.0-9.0, 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 2 shows that HDAC11 is most active in Tricine (pH 8.5), Bicine (pH 8.5) and Tris (pH 9.0) buffers. Since, pH 8.5 would be nearer to the physiological pH and that Tricine (pH 8.5) and Bicine (pH 8.5) show similar results, Bicine (pH 8.5) was chosen for the activity. The results are represented as Fold change in Fluorescence (calculated for the activity obtained at 30 min as compared to that at 0 min for each corresponding buffer, blank with no protein was used for each time point for each buffer separately).

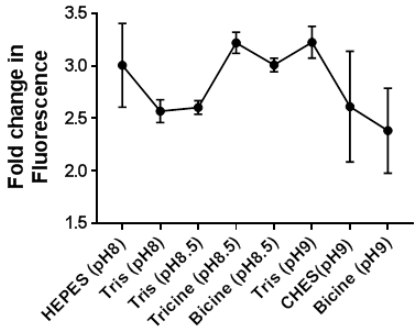

Fig 2. A buffer screen for the activity of HDAC11.