The process of HDAC11 Assay Development: Buffer optimization again

Previously, prior to verification of the linearity and calculation of the Km, different buffers were used in the optimization assays. There the pH range used varied from 8 to 9. This was due to the pI of the protein being 7.3 (*dataset*). But as per literature, the enzyme can show activity at a pH near to its calculated pI. This is because the theoretical pI calculated using the protein sequence can differ significantly from the actual pI of the protein (which can be calculated using isoelectric focusing). This is because the solubility of a protein depends not only the net charge of the protein, but also on the difference in the pK values of the ionizable groups in the folded and unfolded states (Shaw et al., 2001).

Therefore, the buffers were optimized further with an extended pH range of 7.0-8.5.

Table 1 describes the reaction recipe (based on the Km calculated with previous optimization, the substrate concentration was reduced from now on, and also the substrate appears not to be completely soluble at higher concentrations) and Fig 1A-E demonstrates the effect of buffer type and pH on the signals.

Table 1. Reaction recipe for buffer optimization.

7.5 µl Reaction volume	
HDAC11 (µM)	0.125
Boc-Lys-(TFA)-AMC (µM)	100
Assay buffer	20 mM Buffer, pH 7-8.5, 137 mM NaCl, 2.7 mM KCl, 1 mM MgCl2 0.5% BSA (added freshly)
Reaction time at RT (25 ºC)	0 and 30 min
7.5 µl Developer	
Developer conc. (5X stock)	0.1 X
Incubation time	1 hour

As per the results, Tris pH 7.0 will be used for optimization and thus, will be used to replace Bicine pH 8.5. Henceforth, linearity and Km calculation needs to be performed with Tris pH 7.0.

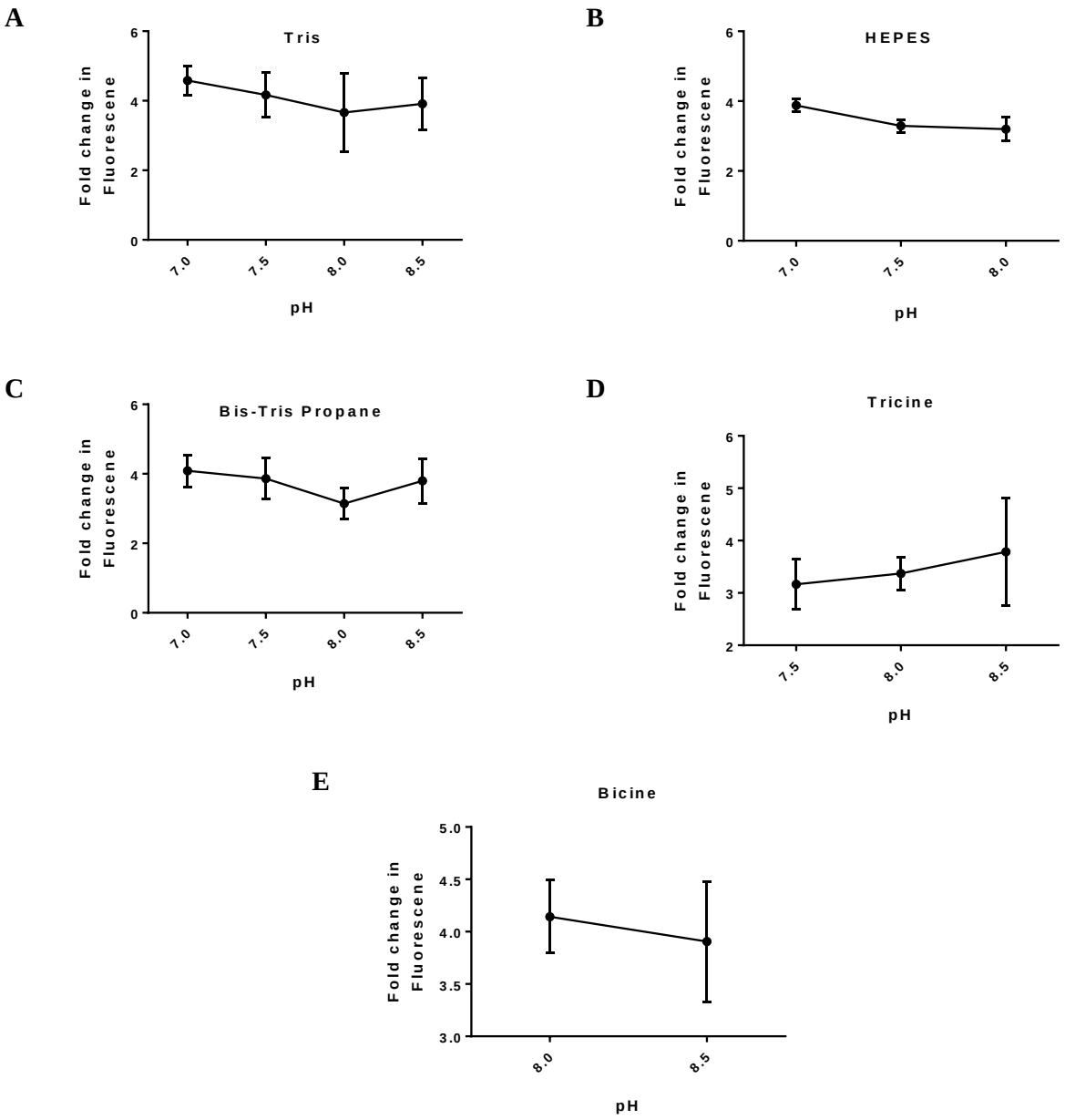

Fig 1. Effect of Buffer type and pH on the activity of HDAC11. (A)-(E) represent the activity of HDAC11 in Tris, HEPES, Bis-Tris Propane, Tricine and Bicine buffers over a period of 30 min.

References:

Shaw, K.L., Grimsley, G.R., Yakovlev, G.I., Makarov, A.A., and Pace, C.N. (2001). The effect of net charge on the solubility, activity, and stability of ribonuclease Sa. Protein science : a publication of the Protein Society *10*, 1206-1215.