

The process of HDAC11 Assay Development: Effect of reducing agent

Following the optimization of buffer again (*dataset*), an effect of adding reducing agent on the enzyme activity was monitored. Accordingly, Table 1 describes the reaction recipe and Fig 1 represents the results for two reducing agents being tested, DTT and TCEP.

Table 1. Reaction recipe for reducing agent.

7.5 μ l Reaction volume	
HDAC11 (μ M)	0.125
Boc-Lys-(TFA)-AMC (μ M)	100
Assay buffer	20 mM Tris, pH 7, 137 mM NaCl, 2.7 mM KCl, 1 mM MgCl ₂ 0.5 mg/ml BSA (added freshly)
Reducing agent (DTT/TCEP)	0-5 mM
Reaction time at RT (25 °C)	0 and 30 min
7.5 μ l Developer	
Developer conc. (5X stock)	0.1 X
Trichostatin A (TSA)	40 μ M
Incubation time	1 hour

Although the signals do not increase drastically, as per the results, 5 mM TCEP was chosen to be added in the assay buffer.

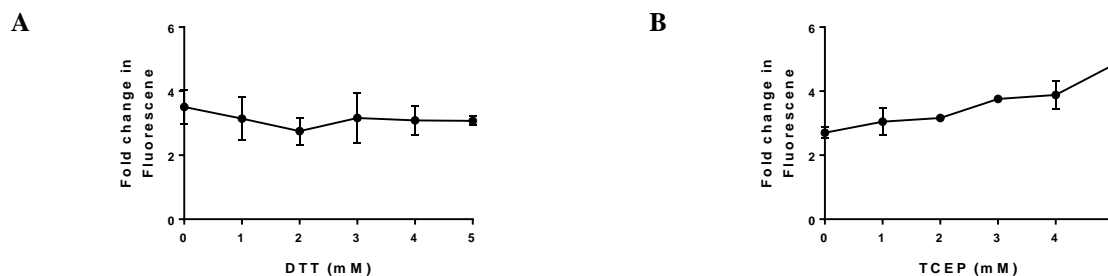


Fig 1. Effect of reducing agents on the activity of HDAC11. (A) DTT and (B) TCEP.