The process of HDAC11 Assay Development: Z'-factor

Since the assay conditions are optimized and no effect of DMSO (till 5%) was observed on the protein activity (*dataset*), the assay could be put in use. As a last step, to check if the assay qualifies for the screening purposes, Z'-factor needs to be calculated (Zhang et al., 1999). To calculate the Z'-factor, the assay is performed and the reaction is set-up in a way that mimics the screening procedure, i.e., a 384-well Axygen black plate is used, wherein half of the plate contained the protein sample, while rest half has no protein (only buffer used as blank). Also, the samples and other reagents (protein, buffer, substrate, developer solution, DMSO) are dispensed using a robotic system (Bravo liquid dispenser). The reaction recipe is described in Table 1.

Table 1. Reaction recipe for Z'-factor calculation.

Reaction components	
Enzyme (HDAC)	HDAC11
Substrate	Boc-Lys-(TFA)-AMC
Mimic for compound	DMSO
Assay buffer	20 mM Tris, pH 7.0, 137 mM NaCl, 2.7 mM KCl, 1 mM MgCl2 0.5 mg/ml BSA (added freshly) 5 mM TCEP
Developer	
Developer conc. (5X stock)	0.1 X
Trichostatin A (TSA)	40 µM
Incubation time	1 hour

Fig 1 is a step-wise representation of the procedure used for calculation of Z'-factor (which would be followed for screening). The concentrations used for the enzyme and substrate are such that in the 15 µl reaction, 250 nM of the enzyme reacts with 48 µM of the substrate. The volume of DMSO being used here (and thus, of the compound when performing screening) is 0.25 µl in a volume of 7.5 µl containing

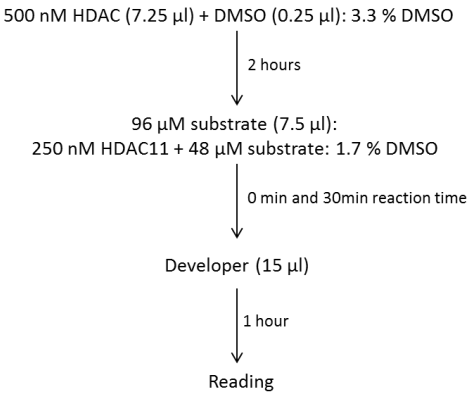

Fig 1. Flow-chart for the procedure of performing Z'-factor calculation.

the enzyme. This is to ensure that the DMSO concentration is as less as possible (below 5% required, higher concentrations can be detrimental for the protein) at any given time in the reaction set-up. The incubation of enzyme and DMSO for 2 hours was performed to mimic the incubation of the compound during screening.

Two identical plates were made corresponding to the two time points being used- 0 and 30 min. Eaxh plate contains protein (half-plate) and no protein (buffer only used as blank, half-plate). Following the reaction, the reading of each well for 0 min was subtracted from the corresponding (same) well of 30 min. These reading obtained following subtraction will be used for Z'-factor calculation.

$$Z'\text{-factor} = 1 - [(3SD \text{ of sample} + 3SD \text{ of blank}) / |(\text{mean of sample} - \text{mean of blank})|]$$

where, SD : standard deviation

| | : absolute value

Fig 2 represents the Z'-factor value for this assay upon calculation using the above formula. A Z'-factor of 0.7 was obtained for this assay.

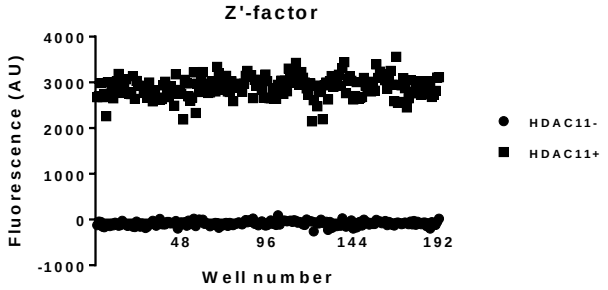

Fig 2. Z'-factor for HDAC11 high-throughput assay.

References:

Zhang, J.H., Chung, T.D., and Oldenburg, K.R. (1999). A Simple Statistical Parameter for Use in Evaluation and Validation of High Throughput Screening Assays. Journal of biomolecular screening *4*, 67-73.