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## The process of HDAC11 Assay Development: Effect of DMSO

For the purpose of compound library screenings, the compounds would be made available in DMSO and thus, when they would be incubated with the protein, the protein solution will now contain DMSO as well. It is important to determine the concentration of DMSO that could be tolerated by the protein such that the protein stays active. Hence, the effect of varying concentration of DMSO was analyzed on HDAC11 activity.

As a follow-up on the process of assay development (dataset), Table 1 describes the reaction recipe.

**Table 1.** Reaction recipe for the effect of DMSO on HDAC11 activity.

| 7.5 μl Reaction volume      |                               |
|-----------------------------|-------------------------------|
| HDAC11 (µM)                 | 0.25                          |
| Boc-Lys-(TFA)-AMC (μM)      | 48                            |
| 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                     |
| Reaction time at RT (25 °C) | 0, 30 min                     |
| 7.5 µl Developer            |                               |
| Developer conc. (5X stock)  | 0.1 X                         |
| Trichostatin A (TSA)        | 40 μΜ                         |
| Incubation time             | 1 hour                        |

Fig 1 shows that no detrimental effect of DMSO occurs on HDAC11 activity for the tested range of 0-5%.

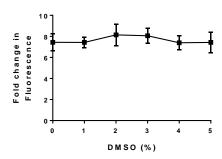


Fig 1. Effect of DMSO on HDAC11 activity.