**Plan for truncated-Sirt3 (118-399) Initial rate in presence of NAM and with and without Honokiol**

|  |  |  |
| --- | --- | --- |
| **For SEC/DLS experiments, SM sent Batch 2B which is 3.5U/uL:**  **1:** Start date is pending approval of this schedule; **2:** Minimum amount of in-house t-Sirt3 (118-399) needed 510U total. **3:** No repeats planned at this time. **4:** three [NAD], 500, 3000, and 15000 uM: Maximum seven [NAM] 0, 100, 200, 2000, 3000, 8000, and 12000 uM and one [Honokiol] = 200 uM. **5:** 600 uM MnSOD [K122], 5U enzyme per reaction. **6:** Three time points 30, 80, and 120 minutes**\*\***. 7: Reactions will be done at 37OC. **TOTAL 102 reactions and TOTAL time required 29 days for the completion of this set.** | | |
| **Task** | **Expected completion date** | **Remarks/Status** |
| **Exp1:** Initial rate with [NAD] 500 uM, [K122] 600 uM, 5% DMSO   * [NAM] 0, 100, 200 uM without Honokiol * [NAM] 0, 100, 200 uM with 200 uM Honokiol * 30, 80, 120 min * Total 18 reactions | Expected to complete in 5 days | 90 Units t-Sirt3 needed |
| **Exp2:** Initial rate with [NAD] 3000 uM, [K122] 600 uM, 5% DMSO   * [NAM] 0, 100, 200, 2000, 3000, 8000, 12000 uM without Honokiol * [NAM] 0, 100, 200, 2000, 3000, 8000, 12000 uM with Honokiol * 30, 80, 120 min * Total 42 reactions | Expected to complete in 12 days which include extensive washing the column as the reactant contains higher concentrations of NAM | 210 Unit t-Sirt3 needed |
| **Exp3:** Initial rate with [NAD] 15000 uM, [K122] 600 uM, 5% DMSO   * [NAM] 0, 100, 200, 2000, 3000, 8000, 12000 uM without Honokiol * [NAM] 0, 100, 200, 2000, 3000, 8000, 12000 uM with Honokiol * 30, 80, 120 min * Total 42 reactions | Expected to complete in 12 days which include extensive washing the column as the reactant contains higher concentrations of both NAD and NAM | 210 Units t-Sirt3 needed |

**\*\*** By looking at slide 2 (raw data), at lower time point (30min), the HPLC readout will be very small at 3000uM NAD/8000uM NAM. We suggest to use 80, 120, 180min as time points for this set of experiments.