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| **The new schedule is made based on the discussion of 8.03.2016 meeting** RC: Specify relation between AU, XG experiments #’s again. Make repeat plan directly analogous to that of AU per latest comments. Assuming triplicate is applied for initial rate experiments. **Triplication for initial rate experiment is required.** |
| **Align AU#** | **Task #** | **Status** | **In-house Enzyme** | **Repeat** |
| **PMC-AU1** | **PMC-XG1-1**: **Deacetylated FdL2 peptide to check the detection limitation of old HPLC****[Pep-3]=0.25, 0.5, 1.0, 1.5 uM** | *7.26. 16 - 7.29. 16 Done. Minimum**[FdL2 peptide] is 3 uM.* | No | Intra and inter-day |
|  | **PMC-XG1-1’: DHP1c solution preparation** To validate how accurate the DHP1c solution concentration made different day for both Citizen MicroBalance and Sartorius Semi-MicroBalance. Weigh different mass of DHP1c sample, then prepare the solution, run on HPLC, find out the linear relationship between peak area and [DHP1c] | *Done* | No | Citizen Micro andSartorius Semi microBalance |
| **PMC-AU2** | **PMC-XG2**: **Deacetylation reactions condition modification within HPLC detection limitation**-**FdL2 peptide.** [FdL2 peptide] = 3uM[NAD+] = 3000uM[Enzo SIRT3 and in-house SIRT3] = 10 UTime point = 5, 30 min | *Done* | 80 U (60ul)**SM previous batch\_6+7 (no FPLC): 1.6U/ul** | Duplicate |
| **PMC-AU3** | **PMC-XG3**: **Deacetylation reactions condition modification within HPLC detection limitation- NAD+.** [FdL2 peptide] = 250uM[NAD+] = 10, 25, 50, 75, 100 uM [Enzo SIRT3 and in-house SIRT3] = 10 UTime point = 5, 30 min | *Done. Minimum [NAD+] is 10 uM.* | 200U(150 ul)**SM batch\_6+7 1.6U/ul** | Duplicate |
| **PMC-AU4** | **PMC-XG4-1**: **DHP2c dose response experiment-HPLC** ***Condition 1:*** [FdL2 peptide] = 3uM [NAD+] = 3000uM***Condition 2:***[FdL2 peptide] = 250 uM[NAD+] = 10 uM***Condition 3:***[FdL2 peptide] = 250 uM [NAD+] = 500uM[DHP2c]=0, 5, 10, 25, 50, 100, 200, 400uM[Enzo SIRT3 and in-house SIRT3] = 10 UTime point = 30 minTotal 3x7x3=63 reactions for triplicate(9 days) | *8.31.16-9.13.16**Done**Note: Was held for the solubility results, which was completed by 8.30.16. The experiments are expecting to start on 8.31.16. DHP2c will be used to replace DHP1c.* | 540U (360 ul)**SM previous batch\_6+7 (no FPLC): 1.6U/ul** | Repeats(N=3/4) |
| **Patent Prep.** | **Patent preparation** | *9.6.16-9.14.16. Done* |
| **PMC-AU4** | **PMC-XG4-2: Standard curve with custom synthesized peptide in HDAC, and desired concentrations of DHP2c in HDAC buffer.** Peptide 3 (deacetylated FdL2 peptide) will be used as standard.1. The standard will be run under 8 different solution conditions (0.46875, 0.9375, 1.875, 3.75, 7.5, 15, 30uM). This will cover the conditions used in endpoint and initial rate experiments.
2. Also, Enzo standard will be used for comparison.
 | *9.15.2016 – 9.19.2016**Done* | No | Duplicate |
| **PMC-AU4** | **PMC-XG4-3**: **DHP1c dose response experiment-FdL assay on TeCan** ***Condition 1:*** [FdL2 peptide] = 3uM**;** [NAD+]=3000uM***Condition 2:*** [FdL2 peptide] = 250 uM; [NAD+] = 10uM.***Condition 3:***[FdL2 peptide] = 250 uM; [NAD+] = 500uM[DHP2c]=0, 5, 10, 25, 50, 100, 200, 400uM.[Enzo/In-house SIRT3] = 10 UTime points = 0, 60 min | *9.20.2016 – 9.26.2016**Done* | 800U (800ul)**SM new FPLC batch****Combined Batch 2 1U/ul** | Duplicate |
| **Literature search** | **Literature search for “Screening assay for sirtuins’ modulator”** | *9.21.2016-9.26.2016**Done* | No other fluorogenic assay is available for our system. |
| **PMC-XG 5****Initial rate Honokiol-in-house SIRT3 in the presence of NAM- Km, Vmax vs. [NAD+]**  | **PMC-XG 5-1: Honokiol dose response experiment-FdL assay on TeCan** ***Condition 1:*** [FdL2 peptide] = 30uM**;** [NAD+]=3000uM***Condition 2:*** [FdL2 peptide] = 250 uM; [NAD+] = 25uM.[DMSO]=5%[Honokiol]=0, 5, 10, 25, 50, 100, 200, 350uM[In-house SIRT3] = 10 UTime points = 0, 60 min | *9.28.2016 – 9.30.2016**Done* | 280U (280ul)**SM new FPLC batch****Combined Batch 2 1U/ul** | Duplicate |
| **PMC-XG 5-2: Honokiol dose response experiment-FdL assay on HPLC** ***Condition 1:*** [FdL2 peptide] = 30uM**;** [NAD+]=3000uM***Condition 2:*** [FdL2 peptide] = 250 uM; [NAD+] = 25uM.[DMSO]=5%[Honokiol]=0, 5, 10, 25, 50, 100, 200, 350uM[In-house SIRT3] = 10 UTime points = 0, 60 min | *10.03.2016 - 10.07.2016**Done* | 280U (280ul)**SM new FPLC batch****Combined Batch 2 1U/ul** | Duplicate |
| **PMC-XG 5-3**: **Optimize suitable NAM concentration for initial rate study on TeCan**[In-house SIRT3]=10 U[FdL2 peptide]=250 uM[NAD+]= 25uM[Honokiol]=200uM [DMSO]=5%[NAM] = 0, 10, 25, 50, 100, 200 uMTime point=0, 60 min | *10.10.2016 - 10.13.2016**Done* | 200U (200 ul)**SM new FPLC batch****Combined Batch 2 1U/ul** | Duplicate |
| **PMC-XG 5-4: Initial rate Honokiol-in-house SIRT3- Km, Vmax vs. [NAD+] on TeCan** [In-house SIRT3]=10 U[FdL2 peptide]=250 uM[NAD+]= 100, 375, 750, 1500, 3000uM[Honokiol]=200uM[DMSO]=0, 5%[NAM] = 25 uMTime point=0, 5, 10, 20, 30, 45, 60, 120min | *10.18.16-10.25.16 One set has been completed.* *Will add another NAM concentration when protein is available.* |
| **In-house SIRT3 Protein purification using FPLC** | *10.19.2016-10.21.2016 1st 4X200ml culture**10.24.2016-10.28.2016 2nd and 3rd 4X200ml culture**10.31.2016-11.2.2016 4th 4X200ml culture**11.3.2016 Stripping and Recharging His-Trap column/ FPLC buffers preparation**11.4.2016 FPLC system wash/equilibrium**11.7.2016 -11.18.2016 4 batches (4X200ml culture per batch) of FPLC purification (Done)* *11.15.2016-11.19.2016 5th and 6th 4X200ml culture (Done)**11.21.2016 -11.22.2016Combine the four individual batches of protein. characterization of new combined batch of protein*  |
|  | **\*Second NAM concentration****[In-house SIRT3]= 10 ul****[NAD+]=375 uM****[FdL2 peptide] = 250 uM****[Honokiol] = 200uM****%DMSO=5%****[NAM]= 15, 25, 35 uM****Time point= 0, 30 min****\*Rapid Quench analysis report****\*Data analysis for MnSOD/SIRT3 system** | *11.23.2016-11.30.2016 (Done)**15uM NAM +200uM HKL provide 74.2% inhibition**35uM NAM+200uM HKL provide 90.6% inhibition**15uM is chosen for next NAM concentration.* |
|  | **\*In-house SIRT3 cell culture (4X800ml Cell Culture)****\*Reach out CRO for SIRTainty experiments. Signed MSA.**  | *12.01.2016-12.08.2016 (Done)**Waiting for SOW from Confluence.* |
|  | **PMC-XG 5-4: Initial rate Honokiol-in-house SIRT3- Km, Vmax vs. [NAD+] on TeCan** [In-house SIRT3]=10 U[FdL2 peptide]=250 uM[NAD+]= 100, 375, 750, 1500, 3000uM[Honokiol]=200uM[DMSO]=5%[NAM] = 0, 15, 25 uMTime point=0, 5, 10, 20, 30, 45, 60, 120min | *12.09.2016, -12.16.2016 Repeat1*(Done)*12.17 - 12.18.2016 data analyses and model fitting (Done)**12.19.2016-12.22.2016 Repeat2 (Done)**12.23.2016 – 12.27.2016 data analyses and model fitting* |
|  | **In-house SIRT3 Protein purification using FPLC for SIRTainty assay** | *01.03.2017 Stripping and Recharging His-Trap column / FPLC buffers preparation**01.04.2017 FPLC system wash/equilibrium**01.05.2017 -01.18.2017 4 batches (4X200ml culture per batch) of FPLC purification* *01.19.2016 -01.20.2016Combine the four individual batches of protein. characterization of new combined batch of protein* |
|  | **PMC-XG-6: Titration of the suitable concentration of Resveratrol** **using SIRTainty assay**[MnSOD K122] = 600uM[NAD+] = 100, 3000 uM[Resveratrol]=0, 5, 10, 25, 50, 100, 500uM. Time point= 30 min. Triplicate is needed. | *01.23.2016-01.25.2016* |