**The following schedule is made base on assuming Triplicate is applied for initial rate experiments. By 8.30.2016, AU will use 3 HPLCs to do Honokiol initial rate experiments for triplicate. XG will move FdL assay up at the time HPLC is not available. When AU finish his work at 10.5.2016, XG will come back to do DHP initial rate work.**

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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] | *8.2.2016–8.8.2016***Proceed with Ascendex Scientific for solubility measurement.** | No | Citizen Micro andSartorius Semi microBalance |
| **PMC-XG1-2**: **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 | *8.09.2016 - 8.12.2016* | 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 | *8.15.2016 – 8.19.2016* | 200U(150 ul)**SM batch\_6+7 1.6U/ul** | Duplicate |
| **PMC-AU4** | **PMC-XG4-1**: **DHP1c does response experiment-HPLC** ***Condition 1:*** [FdL2 peptide] = the lowest [FdL2 peptide] from **PMC-XG1-3** [NAD+] = 3000uM***Condition 2:***[FdL2 peptide] = 250 uM[NAD+] = the lowest [NAD+] from **PMC-XG3**.***Condition 3:***[FdL2 peptide] = 250 uM [NAD+] = 500uM%DMSO = 5%[DHP1c]=0, 50, 75, 100, 200, the maximum DHP1c concentration at 5 % DMSO determined by CRO.[Enzo SIRT3 and in-house SIRT3] = 10 UTime point = 60 min | *8.22.2016 – 8.29.2016* | 540U (360 ul)**SM previous batch\_6+7 (no FPLC): 1.6U/ul** | Triplicate |
|  | **PMC-XG4-2: Standard curve with custom synthesized peptide in assay buffer, 5% DMSO, and desired concentrations of DHP1c in 5% DMSO.** 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, to determine if the addition of NAD and FdL2 peptide influence the results, the following conditions will be performed.
 | *8.30.2016 – 9.2.2016* | No | Triplicate |
|  | **PMC-XG4-3**: **DHP1c does response experiment-FdL assay on TeCan** ***Condition 1:*** [FdL2 peptide] = the lowest [FdL2 peptide] from **PMC-XG1-3;** [NAD+]=3000uM***Condition 2:***[FdL2 peptide] = 250 uM; [NAD+] = the lowest [NAD+] from **PMC-XG3**.***Condition 3:***[FdL2 peptide] = 250 uM; [NAD+] = 500uM%DMSO = 5%[DHP1c]=0, 50, 75, 100, 200, the maximum DHP1c concentration at 5 % DMSO determined by CRO.[Enzo SIRT3 and in-house SIRT3] = 10 UTime points = 0, 60 minControls (These apply for 0, and 60 min time points)The numbers indicate the order how the experiment proceed. For example, Control #E and F is to find out effect of 5% DMSO for the reaction system. #E firstyly adds NAD then peptide 5% DMSO then enzyme then developer then read on TeCan. #F firstyly adds NAD then peptide then enzyme then developer then read on TeCan. After that add 5% DMSO then read on TeCan again. | *9.5.2016 – 9.9.2016* | 540U (360ul)**SM new FPLC batch** | Triplicate |
|  | **PMC-XG 5-1: Initial rate DHP1c-in-house SIRT3- Km, Vmax vs. [NAD+] on TeCan** [In-house SIRT3]=10 U[FdL2 peptide]=250 uM[NAD+]= 100, 500, 1500, 3000uM[DHP1c]=0, ? to be determined from **PMC-XG4-1**%DMSO=0, 5 %Time point=0, 5, 10, 20, 30, 45, 60 min | *9.12.2016 – 9.23.2016* | 4200U (2800ul) **SM new FPLC batch** | Triplicate |
|  | **PMC-XG6-1**: **DHP1c dose response experiment-SIRTainty assay-TeCan** ***Condition 1:*** [Peptide] = the lowest [peptide] from **PMC-XG1-3;**[NAD+] = 3000uM***Condition 2:***[Peptide] = 250 uM;[NAD+] = the lowest [NAD+] from **PMC-XG3**.***Condition 3:***[Peptide] = 250 uM;[NAD+] = 500uM%DMSO = 5%[DHP1c]=0, 50, 75, 100, 200, the maximum DHP1c concentration at 5 % DMSO determined by CRO.[Enzo SIRT3 and in-house SIRT3] = 10 UTime point = 60 min | *9.26.2016 - 10.04.2016* | 360 U (270 ul)**SM new FPLC batch** | Duplicate |
| **PMC-AU5** | **PMC-XG 5-2: Initial rate DHP1c-in-house SIRT3- Km, Vmax vs. [NAD+] on HPLC** [In-house SIRT3]=10 U[FdL2 peptide]=250 uM[NAD+]= 100, 500, 1500, 3000uM[DHP1c]=0, ? to be determined from **PMC-XG4-1**%DMSO=0, 5 %Time point=0, 5, 10, 20, 30, 45, 60 min | *10.06.2016 - 11.17.2016**(3HPLC parallel)* | 4200U (2800ul) **SM new FPLC batch** | Triplicate |
|  | **PMC-XG6-2**: **DHP1c dose response experiment-SIRTainty assay-HPLC** ***Condition 1:*** [Peptide] = the lowest [peptide] from **PMC-XG1-3**[NAD+] = 3000uM***Condition 2:***[Peptide] = 250 uM; [NAD+] = the lowest [NAD+] from **PMC-XG3**.***Condition 3:***[Peptide] = 250 uM; [NAD+] = 500uM%DMSO = 5%[DHP1c]=0, 50, 75, 100, 200, the maximum DHP1c concentration at 5 % DMSO determined by CRO.[Enzo SIRT3 and in-house SIRT3] = 10 UTime point = 60 min | *11.18.2016-11.25.2016* | 360 U (270 ul)**SM new FPLC batch** | Duplicate |
| **Total in-house enzyme needed:** * AU will use the first big batch of purified enzyme (FPLC).
* Before 8.28.2016, XG will use SM previous batch 6+7 (1.6U/ul) for the experiments. These batches were using gravity column not FPLC. Their activities are comparable.
* After new purified enzyme is available, 6500 ul will be needed if we include SIRTainty assay. If we do not include SIRTainty assay, 5960 ul is needed assuming the activity of purified enzyme is 1.5U/ul.
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**The following schedule is made base on assuming Duplicate is applied for initial rate experiments. By 8.30.2016, AU will start doing Honokiol initial rate experiments. Since duplicate is required, AU will occupy 2 HPLCs, and XG can use one HPLC for DHP initial rate work. By the time AU finish Honokiol work, and another HPLC will be available, XG will do the duplicate. Then go back to FdL assay.**

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| **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] | *8.2.2016–8.8.2016***Proceed with Ascendex Scientific for solubility measurement.** | No | Citizen Micro andSartorius Semi microBalance |
| **PMC-XG1-2**: **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 | *8.09.2016 - 8.12.2016* | 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 | *8.15.2016 – 8.19.2016* | 200U(150 ul)**SM batch\_6+7 1.6U/ul** | Duplicate |
| **PMC-AU4** | **PMC-XG4-1**: **DHP1c does response experiment-HPLC** ***Condition 1:*** [FdL2 peptide] = the lowest [FdL2 peptide] from **PMC-XG1-3** [NAD+] = 3000uM***Condition 2:***[FdL2 peptide] = 250 uM[NAD+] = the lowest [NAD+] from **PMC-XG3**.***Condition 3:***[FdL2 peptide] = 250 uM [NAD+] = 500uM%DMSO = 5%[DHP1c]=0, 50, 75, 100, 200, the maximum DHP1c concentration at 5 % DMSO determined by CRO.[Enzo SIRT3 and in-house SIRT3] = 10 UTime point = 60 min | *8.22.2016 – 8.29.2016* | 540U (360 ul)**SM previous batch\_6+7 (no FPLC): 1.6U/ul** | Triplicate |
| **PMC-AU5** | **PMC-XG 5-2: Initial rate DHP1c-in-house SIRT3- Km, Vmax vs. [NAD+] on HPLC** [In-house SIRT3]=10 U[FdL2 peptide]=250 uM[NAD+]= 100, 500, 1500, 3000uM[DHP1c]=0, ? to be determined from **PMC-XG4-1**%DMSO=0, 5 %Time point=0, 5, 10, 20, 30, 45, 60 min | *8.30.2016 - 10.11.2016**(one HPLC-single)* *10.12.2016 – 11.16.2016**(one HPLC-Duplicate)* | 4200U (2800ul) **SM new FPLC batch** | Duplicate |
|  | **PMC-XG4-2: Standard curve with custom synthesized peptide in assay buffer, 5% DMSO, and desired concentrations of DHP1c in 5% DMSO.** 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, to determine if the addition of NAD and FdL2 peptide influence the results, the following conditions will be performed.
 | *11.17.2016 – 11.22.2016* | No | Triplicate |
|  | **PMC-XG4-3**: **DHP1c does response experiment-FdL assay on TeCan** ***Condition 1:*** [FdL2 peptide] = the lowest [FdL2 peptide] from **PMC-XG1-3;** [NAD+]=3000uM***Condition 2:***[FdL2 peptide] = 250 uM; [NAD+] = the lowest [NAD+] from **PMC-XG3**.***Condition 3:***[FdL2 peptide] = 250 uM; [NAD+] = 500uM%DMSO = 5%[DHP1c]=0, 50, 75, 100, 200, the maximum DHP1c concentration at 5 % DMSO determined by CRO.[Enzo SIRT3 and in-house SIRT3] = 10 UTime points = 0, 60 minControls (These apply for 0, and 60 min time points)The numbers indicate the order how the experiment proceed. For example, Control #E and F is to find out effect of 5% DMSO for the reaction system. #E firstyly adds NAD then peptide 5% DMSO then enzyme then developer then read on TeCan. #F firstyly adds NAD then peptide then enzyme then developer then read on TeCan. After that add 5% DMSO then read on TeCan again. | *11.23.2016 – 11.28.2016* | 540U (360ul)**SM new FPLC batch** | Triplicate |
|  | **PMC-XG 5-1: Initial rate DHP1c-in-house SIRT3- Km, Vmax vs. [NAD+] on TeCan** [In-house SIRT3]=10 U[FdL2 peptide]=250 uM[NAD+]= 100, 500, 1500, 3000uM[DHP1c]=0, ? to be determined from **PMC-XG4-1**%DMSO=0, 5 %Time point=0, 5, 10, 20, 30, 45, 60 min | *11.29.2016 – 12.05.2016* | 4200U (2800ul) **SM new FPLC batch** | Triplicate |
|  | **PMC-XG6-1**: **DHP1c dose response experiment-SIRTainty assay-TeCan** ***Condition 1:*** [Peptide] = the lowest [peptide] from **PMC-XG1-3;**[NAD+] = 3000uM***Condition 2:***[Peptide] = 250 uM;[NAD+] = the lowest [NAD+] from **PMC-XG3**.***Condition 3:***[Peptide] = 250 uM;[NAD+] = 500uM%DMSO = 5%[DHP1c]=0, 50, 75, 100, 200, the maximum DHP1c concentration at 5 % DMSO determined by CRO.[Enzo SIRT3 and in-house SIRT3] = 10 UTime point = 60 min | *12.06.2016 - 12.09.2016* | 360 U (270 ul)**SM new FPLC batch** | Duplicate |
|  | **PMC-XG6-2**: **DHP1c dose response experiment-SIRTainty assay-HPLC** ***Condition 1:*** [Peptide] = the lowest [peptide] from **PMC-XG1-3**[NAD+] = 3000uM***Condition 2:***[Peptide] = 250 uM; [NAD+] = the lowest [NAD+] from **PMC-XG3**.***Condition 3:***[Peptide] = 250 uM; [NAD+] = 500uM%DMSO = 5%[DHP1c]=0, 50, 75, 100, 200, the maximum DHP1c concentration at 5 % DMSO determined by CRO.[Enzo SIRT3 and in-house SIRT3] = 10 UTime point = 60 min | *12.09.2016-12.20.2016* | 360 U (270 ul)**SM new FPLC batch** | Duplicate |
| **Total in-house enzyme needed:** * AU will use the first big batch of purified enzyme (FPLC).
* Before 8.28.2016, XG will use SM previous batch 6+7 (1.6U/ul) for the experiments. These batches were using gravity column not FPLC. Their activities are comparable.
* After new purified enzyme is available, 6500 ul will be needed if we include SIRTainty assay. If we do not include SIRTainty assay, 5960 ul is needed assuming the activity of purified enzyme is 1.5U/ul.
 |

Footnote:

1. For duplication, DHP initial rate experiment (HPLC) will be moved up and start at 8.30.2016. By this time we are expected to have the 3rd HPLC under working condition. The initial rate experiment will be firstly done once (no repeat). The first set experiments will be completed on 10.11.2016. Then the duplication will be started as soon as the other HPLC is available, which will be 10.3.2016 (completion of AU initial rate experiment). The duplicate will be finished on 11.16.2016. As suggested the triplicate will be done either in house at later time or by CRO.
2. In the revised AU/XG schedules, there is no conflict of usage of HPLC. AU will use one HPLC for expt. PMC-AU4, which aligned to XG’s.