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| --- | --- | --- | --- |
| **Experiment #** | **Experiment name** | **Type** | **Comments** |
| **1** | Sirt3 + NAD ***(titrate)*** | **Positive Controls** | To determine **NAD** binding affinity only |
| **2** | Sirt3 + Ac-MnSOD ***(titrate)*** | To determine **Ac-MnSOD** binding affinity only |
| **3** | Sirt3 + DeAc-MnSOD ***(saturating)*** + NAD ***(titrate)*** | To determine **NAD** binding affinity in presence of **saturating product (DeAc-MnSOD)** |
| **4** | Sirt3 + “modulator” (SM) ***(titrate)*** | **Co-binding experiments with saturating substrates/product and “modulator” (SM)** | To determine binding affinity of **SM** only |
| **5** | Sirt3 + Ac-MnSOD ***(saturating)*** + “modulator” (SM) ***(titrate)*** | To determine binding affinity of **SM** in presence of **saturating peptide substrate** |
| **6** | Sirt3 + NAD ***(saturating)*** + “modulator” (SM) ***(titrate)*** | To determine binding affinity of **SM** in presence of **saturating NAD substrate** |
| **7** | Sirt3 + NAD ***(saturating)*** + DeAc-MnSOD ***(saturating)*** + “modulator” (SM) ***(titrate)*** | To determine binding affinity of **SM** in presence of **saturating NAD substrate** and **saturating product peptide** |

**Legend:**

1. **Sirt3:** Human Sirtuin 3 (protein to be tested)
2. **NAD:** Nicotinamide adenine dinucleotide (ligand/substrate)
3. **Ac-MnSOD:** Acetylated MnSOD peptide (ligand/substrate)
4. **DeAc-MnSOD:** Deacetylated MnSOD peptide (ligand/product)
5. **“Modulator” (SM):** (ligand) – **We are interested in measuring the binding affinity of this modulator to Sirt3.**

**\*\* Important: Experiment # 4 (Sirt3 + “modulator” (SM)) is the high priority experiment** (it would be great if this could be done by the 1st week of September).