SIRTainty™ Class III HDAC Assay

Catalog No. 17-10090

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Not for use in diagnostic procedures

USA & Canada Phone: +1(800) 645-5476
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Italy: 848.845.645
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Introduction

Posttranslational acetylation of lysines occurs in a wide variety of proteins, and consequently regulates a diverse array of intermolecular interactions and biological processes. Proteomic analysis of lysine acetylation in mammalian cells has revealed >3600 acetylated lysine sites occurring on >1700 proteins. This diverse array of proteins function in many major cellular processes, including gene regulation, cytoskeletal organization, protein trafficking, and cell signaling (1). On histone proteins, the universe of lysine acetylation is controlled by the opposing enzymatic activities of histone acetyltransferases (HAT) and histone deacetylases (HDAC). Given the diversity of lysine acetylated proteins and processes in which they occur, modulators of HATs and HDACs are major targets for drug discovery efforts in a number of disease processes, including metabolism, cancer and cardiovascular disease.

Four different classes of histone deacetylases have been described. The first two classes are HDACs which are inhibited by trichostatin A (TSA). The third group (Class III HDAC's) is NAD+-dependent proteins not inhibited by TSA. The fourth classes of HDACs are atypical and are considered HDAC's due to DNA sequence similarity to the other classes of HDAC's.

Class III HDAC's, also known as sirtuins, are mechanistically distinct from class I and class II HDACs in that they couple deacetylation of the peptide/protein substrate to cleavage of NAD^+ to form nicotinamide and O-acetyl-ADP-ribose. The first member of the sirtuin family to be assigned a physiological role was the yeast Sir2, which was shown to increase mother cell life span when over expressed. The mammalian sirtuins comprise 7 members, termed Sirt1-7, which share the NAD^+-binding catalytic domain, but differ in N- and C-termini, subcellular localization, substrate preference, and ultimately in biological function. For example, Sirt1 resides primarily in the nucleus, where it deacetylates several transcriptional regulators, including PGC-1α to promote mitochondrial biogenesis and gluconeogenesis, and p53 to reduce apoptosis. Sirtuins 3-5 are located in mitochondria, and Sirt3 has been shown to deacetylate several metabolic enzymes to divert alternative carbon sources to the TCA cycle (reviewed in reference 2).

Multiple lines of evidence have suggested that the activation of sirtuins in vivo could represent therapeutic target for the maintenance of metabolic homeostasis (3, 4, 5) Subsequent studies searching for activators of sirtuin activity indicated the plant polyphenol resveratrol as a potential activator of Sirt1 (6, 7) However, these studies were called into question as a result of data demonstrating that resveratrol-mediated activation of sirtuins was actually due to an artifact of the fluorescently labeled peptide substrate used in the commercially available assay used for these studies (8).

The SIRTainty approach (see figure 1 page 3) eliminates artifacts due to resveratrol and potential interference of enzyme substrate interactions due to the presence of bulky fluorophores. Unlike other sirtuin assays that utilize a fluorescently tagged acetylated peptide substrate, the SIRTainty™ assay employs an untagged acetylated peptide substrate. This approach not only enables unparalleled flexibility in your choice of sirtuin isoform and peptide substrate, but also helps avoid potential artifacts attributed to the use of a fluorescently labeled substrate (9).

SIRTainty Assay Kit Overview

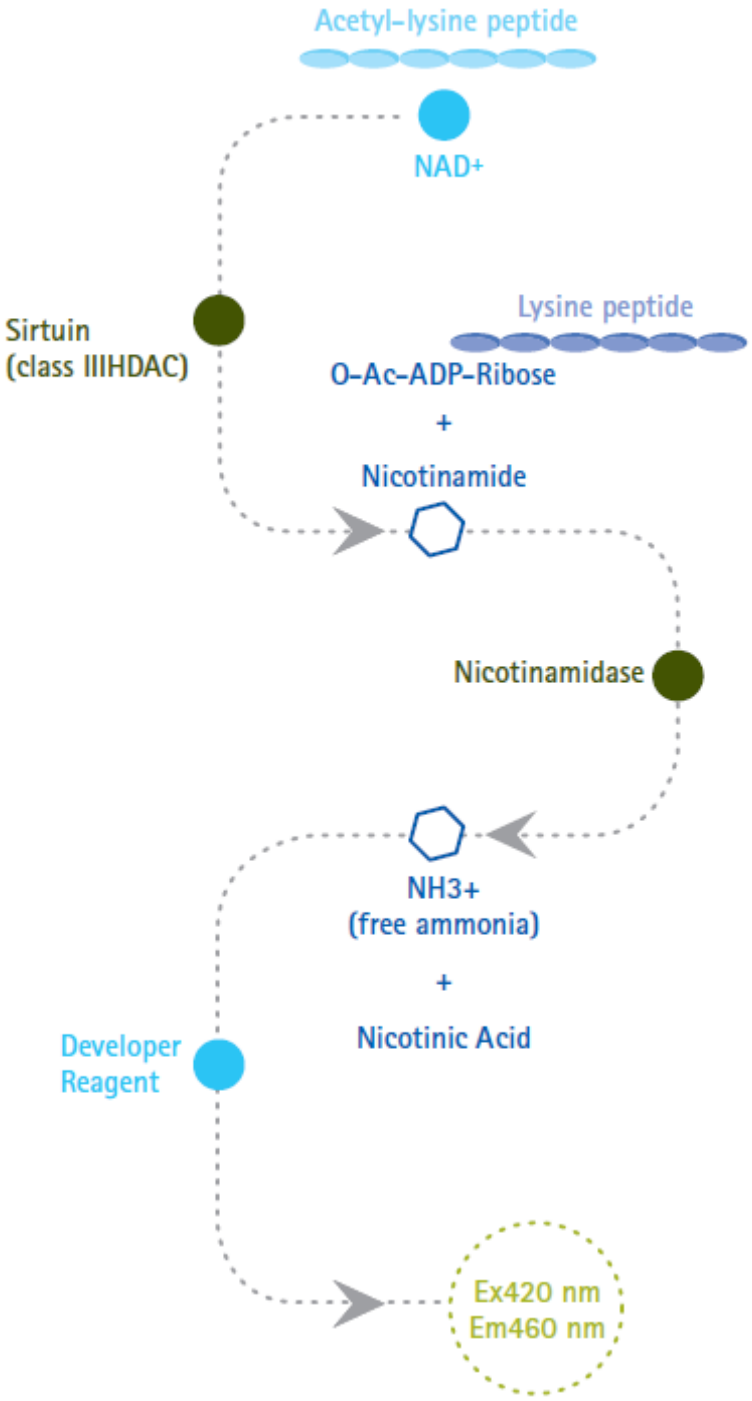
The SIRTainty™ Class III HDAC Assay (Cat. # 17-10090) is a flexible and reliable homogeneous no wash assay for quantifying sirtuin activity. Based upon novel published (10), and patent pending technology, this easy to perform assay employs nicotinamidase to measure nicotinamide generated upon cleavage of NAD^+ during sirtuin mediated deacetylation of a substrate (see figure 1 on page 3) providing a direct assessment of the activity of class III HDAC enzymes.

To perform the SIRTainty assay, a sirtuin enzyme, β-NAD, acetylated peptide substrate, test compound, and nicotinamidase enzyme are combined and incubated for 30 minutes. During this time the acetylated peptide substrate is acted upon by the sirtuin enzyme to produce nicotinamide. In a secondary reaction, the nicotinamidase enzyme converts the nicotinamide into nicotinic acid and NH_3^+ (free ammonia). To generate a signal for readout, a proprietary developer reagent is added and the signal is read using a fluorescent plate reader.

The robust performance of this assay makes it appropriate for measuring sirtuin activity as well as for screening of activators and inhibitors of sirtuin enzymes. This kit contains sufficient components to run 96 assays including standards and controls. The format of the SIRTainty assay provides flexibility in your approach to assay design to allow the analysis of different substrates, sirtuin enzymes, and inhibitors. For example, this kit includes sufficient peptide substrate for all 96 assays. However, other acetylated substrates can be used in place of the acetylated peptide substrate provided with this kit.

The SIRTainty assay is designed to allow the analysis sirtuins 1-7 and has been rigorously validated on sirtuins 1-3 (see figure 2 page 10). A recombinant Sirt1 enzyme is provided for use as a positive control and for the evaluation of test compounds. The amount provided is sufficient to allow the processing of an entire 96-well plate containing a set of test samples, standards and controls using 1.5 units of enzyme per assay. Should your experiment require larger amounts of Sirt1 or other sirtuin family members, additional recombinant Sirt1, Sirt2, and Sirt3 as well as a variety of sirtuin inhibitors are available separately. Please see “Additional Materials” section on pages 4-5 for ordering information.

Figure 1. SIRTainty Assay Flow Chart



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Kit Components

SIRTainty Class III HDAC Assay (Cat# 17-10090) Store components at -80º C Plates and covers can be stored at room temperature (18-25º C)		
Component	**Part #**	**Quantity**
Black 96 well plate	R5013	1 plate
SIRTainty™ Assay Buffer	CS207792	10 mL
Plate Covers	3006581	2 plate covers
Acetylated Peptide Substrate	CS207790	1 vial (75 µL at 1 mM)
β-NAD	CS207788	1 vial (30 µL at 50 mM)
Nicotinamide (NAM)	CS207786	1 vial (10 µL of at 10 mM)
Recombinant Sirt1 Enzyme *See label for unit definition*	CS207782	1 vial containing 120 units
Recombinant Nicotinamidase Enzyme with 50% glycerol.	CS207784	1 vial (60 µg at 1 mg/mL)
Suramin (Sirt Inhibitor) in ddH_2O	CS207778	1 vial (150 µL at 25 mM)
Developer Reagent	CS207780	4 mL at 10 mM.

Additional Materials Required But Not Provided

- Multi-channel or repeating pipettes
- Plate shaker
- Pipettors and tips capable of accurately measuring 1-1000 µL
- Graduated serological pipettes
- 96-well microtiter plate reader with fluorescence readout.
- Graphing software for plotting data or graph paper for manual plotting of data
- Microcentrifuge tubes for standard and sample dilutions
- Mechanical vortex
- 1 liter container
- Distilled or deionized water
- Appropriate solvents (i.e. DMSO) to dissolve inhibitor compounds.

Calbiochem® Brand Related and Accessory Products

- SIRT1, GST-Fusion, Human, Recombinant, *E. coli* cat# 524743
- SIRT2, His•Tag®, Human, Recombinant, *E. coli* cat# 524744
- SIRT3, GST Fusion, Human, Recombinant, *E. coli* cat# 524745
- Suramin, Sodium Salt cat# 574625
- SIRT1/2 Inhibitor IV, Cambinol cat# 566323
- SIRT1/2 Inhibitor VII cat# 566327

- SIRT1/2 Inhibitor VIII, Salermide; cat# 566330
- SIRT1 Inhibitor III cat# 566322
- SIRT1 Inhibitor IV, (S)-35 cat# 566325
- SIRT2 Inhibitor, AGK2 cat# 566324
- SIRT2 Inhibitor II, AK-1 cat# 566331
- Sirtinol cat# 566320
- InSolution™ Sirtinol cat# 566321

Storage and Stability

Maintain the unopened kit (17-10090) at -80ºC. Unused components should be stored at -80ºC. When stored properly this kit is good for up to 6 months after date of receipt. Avoid repeated freeze/thaw cycles, aliquot if necessary.

Hazards and Precautions

- This kit is designed for research use only. It is not intended for use in humans or animals.
- All chemicals should be considered potentially hazardous and principles of good laboratory practice should be followed.
- Wear glove and use PPE (personal protective equipment) as appropriate.
- **Important:** Developer Reagent is light sensitive. Protect from exposure to bright light.

Important Technical Notes

- Please read the entire manual before using this kit for the first time. Important information on reagent preparation and handling as well as guidelines for customizing and optimizing the reaction for user-provided peptide substrates, enzymes, and test compounds are outlined below and in the appendix. It is important to review this information and carefully design your experiment before running your assay.
- The protocol provided has been designed to optimize the kit's performance. Deviation from the instructions may result in suboptimal performance of the kit and inaccurate data.
- All reagents should be rapidly thawed just prior to use and kept on ice until ready to use.
- Promptly returned unused reagents to appropriate storage condition.
- Do not mix or interchange reagents from various kit lots.
- Vials containing less than 150 µL of material should be spun briefly in a microcentrifuge to settle the contents and ensure that no droplets are present on the cap.

Reagents Preparation – Required before starting assay

1. **Suramin and Other Test compounds.** For the provided Suramin (Sirt inhibitor) use SIRTainty™ Assay Buffer to generate 10-fold serial dilutions that give a final concentration of 0-1mM. For a greater than 75% inhibition of Sirt1use >100 uM of Suramin. For other compounds, determine a suitable solvent to dissolve the test compounds to create a concentrated stock. For most compounds DMSO is an appropriate solvent. It is the responsibility of, the user to determine the most appropriate solvent and effective concentration. After creating the stock solution, make further dilutions with SIRTainty Assay Buffer. To avoid potential enzyme inhibition, the final DMSO concentration should be no more than 2% in the final reaction. If other organic solvents are used, the impact of the solvent on assay performance should be determined.

2. **Acetylated peptide substrate solution.** Briefly spin vial to settle contents prior to opening. Reagent is provided as a 1 mM concentrated stock. The final concentration used in the standard protocol is 25 µM. Prepare a fresh 5X solution using the SIRTainty™ Assay Buffer (CS207792) to dilute the concentrated Acetylated Peptide Substrate provided with the kit. Keep the diluted peptide substrate on ice until ready to use.

   Example: For a final substrate concentration of 25 µM, prepare a 125 µM (5X) working solution by diluting the stock solution (CS207790) 1:8 in SIRTainty™ Assay Buffer. Vortex for 10 seconds at medium speed to mix well and keep on ice until use.

3. **Nicotinamide (NAM) standard.** Briefly spin vial to settle contents prior to opening. To create a standard curve for quantitative analysis of your assay, prepare a NAM 2-fold dilution series dilution from 25 µM to 0 µM.
   i) 1st point: 5X solution as 125 µM Dilute stock NAM (CS207786) 1:80 in SIRTainty Assay Buffer (1 µLNAM stock solution in 79 µL assay buffer), and vortex for 10 seconds at medium speed to mix well.
   ii) 2nd point: 5X solution as 62.5 µM 2-fold serial dilution from 1st point, 40 µL of 1st point in 40 µL assay buffer, mix well as the 1st point solution.
   iii) Repeat step 2 to create a 7 point standard curve as shown in Table 1 below.
   iv) Use 40µL of assay buffer to create an 8th point for a background control.

Table 1. NAM Standard Curve

Sample	5X solution concentration (µM)	Final Concentration (µM)
1	125	25
2	62.5	12.5
3	31.25	6.25
4	15.63	3.123
5	7.81	1.56
6	3.91	0.78
7	1.95	0.39
8	0	0

4. **Recombinant Nicotinamidase**. Briefly spin vial to settle contents prior to opening. Just prior to starting the assay, dilute the 1 mg/mL Recombinant Nicotinamidase stock solution (CS207784) to 0.1mg/mL with SIRTainty™ Assay Buffer (CS 207792). For 100 reactions, prepare 500 µL Nicotinamidase working solution by adding 50 µL stock Nicotinamidase to 450 µL assay buffer and vortexing for 10 seconds at medium speed to mix well. Keep on ice until ready to use.

5. **β-NAD solution.** Briefly spin vial to settle contents prior to opening. Prepare a fresh dilution of the 50 mM stock β-NAD solution (CS207788) to 5X of the desired final concentration (0.2 mM for standard protocol) with SIRTainty Assay Buffer (CS207792), and keep on ice until ready to use.

   Example: For a final concentration of 0.2 mM β-NAD, prepare a 1 mM (5X) working solution by diluting the β-NAD stock solution 1:50 in SIRTainty Assay Buffer. Vortex for 10 seconds at medium speed to mix well, and keep on ice until ready to use.

6. **Recombinant Sirt1 Enzyme.** Briefly spin vial to settle contents prior to opening. Refer to the label of the provided Recombinant Sirt1 Enzyme (CS207782) and determine the units per µL. Use SIRTainty Assay buffer ((CS207792) to dilute this enzyme to create a 5X stock (typically 1 Unit/µL).

   Example: To use 5 units of Sirt1 per reaction, prepare a 1 Unit/µL working solution by diluting the enzyme stock in SIRTainty assay buffer. Vortex for 10 seconds at medium speed to mix well. Keep on ice until ready to use.

   Note: 120 units of Sirt1 enzyme is provided with this kit. This enzyme can be used as a positive control or as an enzyme for compound or substrate activity screening. The amount of enzyme provided allows the analysis of 80 samples plus 24 standards and 2 negative controls using 1.5 units of enzyme per reaction or 24 samples plus 24 standards and 2 controls at 5 units per reaction. This assay is designed to work with all known class II HDACs. Additional Sirt1 enzyme, as well as recombinant Sirt2 and Sirt3 are available. See pages 4-5 “Accessory Products” for ordering information.

7. **Developer reagent.** Warm the ready-to-use developer reagent (CS207780) at 37ºC for about 15 minutes prior to adding to the reaction samples. If a precipitate is present, continue incubating and swirling the tube until reagent completely solubilized. Once the reagent has been solublized it can remain at room temperature, protected from light until ready for use.

Standard Assay Protocol

Important: Before starting this assay, please read and follow instructions in the preceding sections. The reagents provided in this kit are designed and optimized to run the standard assay protocol. This protocol is ideal for testing of inhibitor/activators of sirtuin activity, evaluation of substrates, and testing of sirtuin mutant activity. If alternate sources and combinations of enzymes, or unique substrates are used reagent titration may be required. If using alternate sources of reagents, for optimal results please see page 12 “Guidelines for Assay Optimization”.

I. Setup a NAM standard curve with provided acetylated peptide substrate.

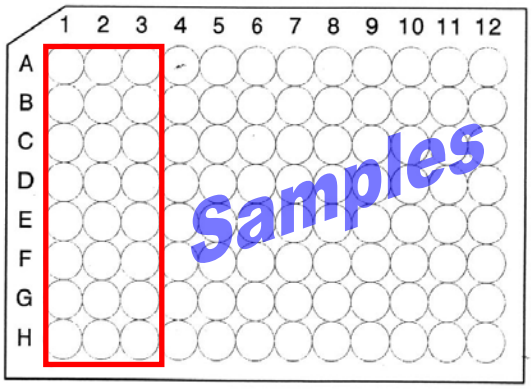
As shown in the figure below, set-up 3 columns of NAM standard (in triplicate) using the NAM serial dilutions prepared in the previous section. . Cover unused wells with plate sealer.

1. Label 8 test tubes #1-8 and mark #8 as “0 dose”. Prepare as described in “Reagents Preparations” section – see page 6 Step 3.
2. Dispense 5 µL/well of the standard solutions made above.

Standard Curve Layout:

NAM Standard *(µM)*

1) 25
2) 12.5
3) 6.25
4) 3.125
5) 1.56
6) 0.78
7) 0.391
8) 0



II. Setup sirtuin activity samples. Compounds can be added to the sirtuin reactions in a dose-response curve to determine IC_{50} value.

3. Setup sirtuin reactions for controls, test sample, standards and buffer only background control and outlined in Table 2.

Table 2.

Sirtuin Reaction	Negative	Positive	Inhibitor/ Compounds	NAM Standard	Buffer only
Inhibitor/Compounds	-	-	5 µL	-	-
Acetylated Peptide Substrate (125µM)	5 µL	5 µL	5 µL	-	-
Sirtuin (1 Unit/µL)	-	5 µL	5 µL	-	-
β-NAD (1 mM)	5 µL	5 µL	5 µL	-	-
Nicotinamidase (0.1 µg/µL)	5 µL	5 µL	5 µL	5 µL	-
NAM standard as in table 1.	-	-	-	5 µL	-
SIRTainty Assay Buffer	10 µL	5µL	-	15 µL	25 µL

4. For reactions with test compounds, combine sirtuin enzyme and test compounds, and incubate for 10-15 minutes at room temperature. Then add the other reagents to the final reaction mix to obtain a total volume of 25 µL.

5. Gently tap the plate to mix the solutions, and cover with one plate sealer. Incubate for 30 minutes at 30-37°C on a plate shaker with gentle agitation (e.g. Labnet, Vortemp 56™ EVC temperature controlled plate shaker at 45 rpm).

6. At least 20 minutes prior to completion of the reaction in step 5, prepare the developer reagent as described in "Reagents Preparation" section page 7 step 7.

   Remember: Developer Reagent is light sensitive; protect from bright light.

7. Pipet 25 µL developer reagent to each well under dimmed light. Gently tap to mix the solutions, cover wells with plate sealer and wrap the 96-well plate in aluminum foil to protect from light. Incubate for 30 minutes at room temperature on a plate shaker with gentle agitation (e.g. Lab-line Instruments, Titer plate shaker at set at speed 2).

8. Read the fluorescence on a fluorometer with filters set to excitation ~420nm +/-20, and emission ~450nm (+/- 20). A 0.1s or 1s read is generally recommended.

   Guideline Instrument Parameters:

   A). Molecular Devices Analyst® Plate Reader. For complete details please refer to parameters recommended in the instrument instruction manual:

Excitation	405 nm +/- 20
Emission	460 nm +/- 40
Integration time	50 µsec

   B). Perkin-Elmer VICTOR2™. For complete details please refer to parameters recommended in the instrument instruction manual:

Excitation	405 nm
Emission	460nm
Measurement time	1sec
CW-lamp energy	10000

9. Data can be interpreted by GraphPad PRISM® nonlinear fit, sigmoidal dose-response (variable slope). Alternatively, similar data analysis software such as SigmaPlot®, Origen®, etc. can be used.

Appendix

Section I Examples of Assay Results

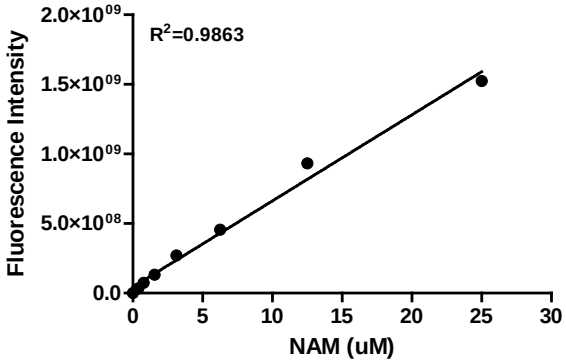


Figure 1. Standard curve of Nicotinamide (NAM)
Nicotinamide from 0~25 µM was incubated with 0.5 µg nicotinamidase for 30 minutes. Fluorescent signal was determined after incubation with 10 mM developer reagent for 30 minutes at room temperature, and a linear curve fit was applied.

NOTE: Standard curve data presented above for reference use only. This data should not be used to interpret your assay results. A standard curve must be generated for each assay.

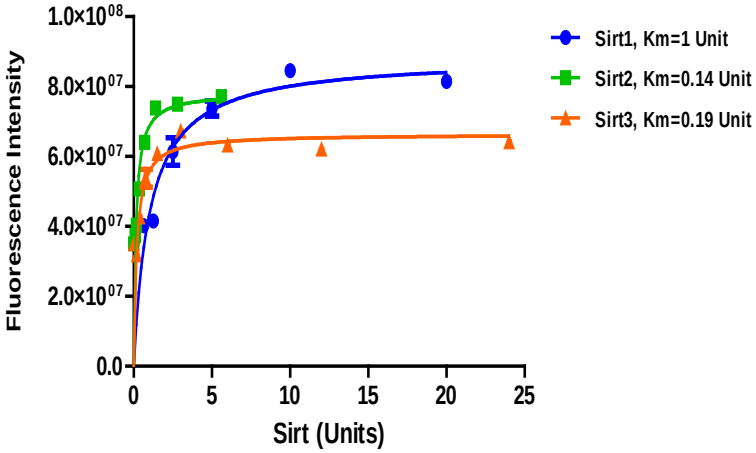


Figure 2. Determination of Sirtuin K_m values
Sirtuins 1, 2, and 3 at a range of concentrations were incubated with 25 µM acetylated peptide substrate and 0.2 mM β-NAD using the SIRTainty™ assay. The K_m value was determined by non-linear curve fit of Michaelis-Menten. This value may be referenced when planning assays with test compounds.

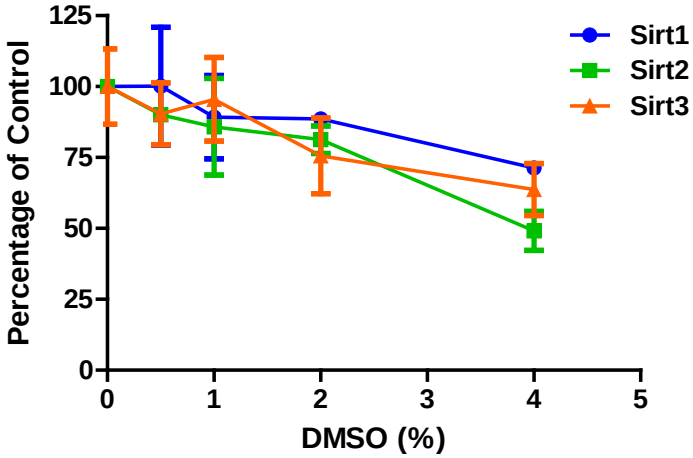


Figure 3. DMSO Effects on Sirtuins.
Recombinant Sirt1 (5 Units), Sirt2 (0.7 Units) and Sirt3 (1.5 Units) were incubated with 25 µM substrate and 0.2 mM β-NAD using SIRTainty assay. A range of DMSO (0-4%) was tested along with the Sirt reactions. Up to 2% final DMSO was tolerated and thus represents the maximum that should be present during analysis of test compounds

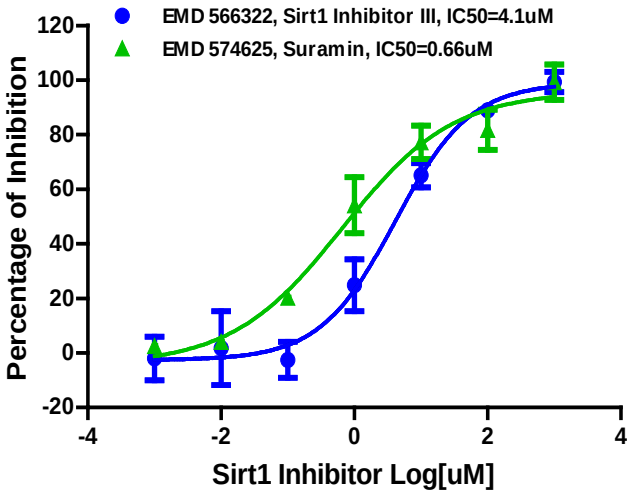


Figure 4. Determination of Sirt1 Inhibitor IC_{50} values

Recombinant Sirt1 (5 Units) was incubated with 50 µM substrate, 0.2 mM β-NAD, and either Suramin (included) or Sirt1 Inhibitor III (available separately, Calbiochem part# 566322). Data was analyzed by non-linear curve fit with sigmoidal dose response with variable slope.

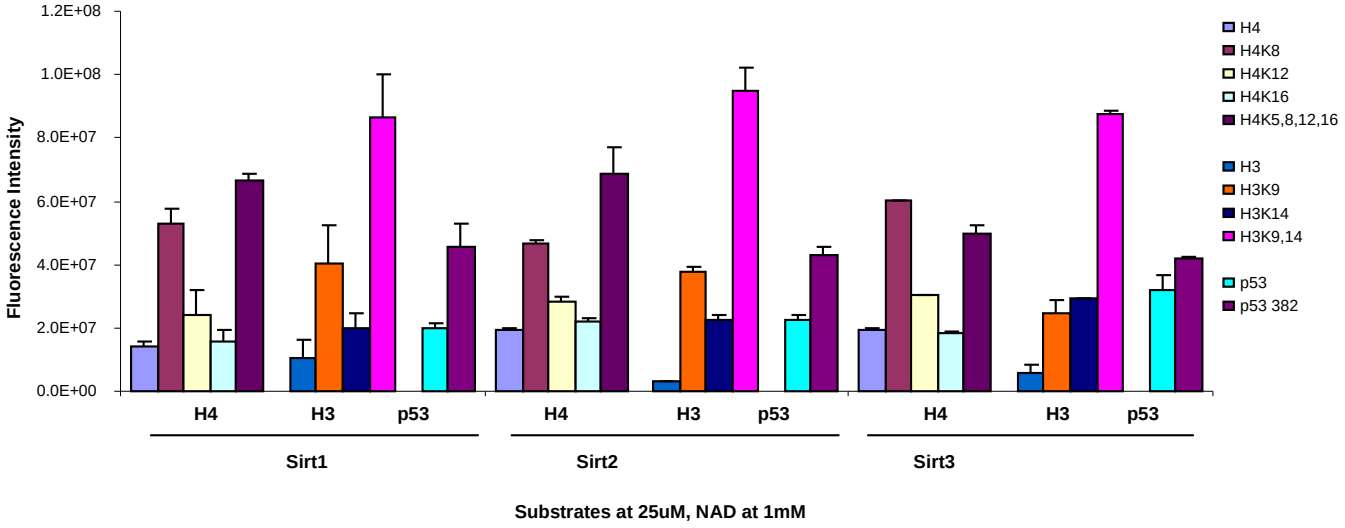


Figure 5. Activity of Sirt1, 2, and 3 with a panel of Acetylated Peptide Substrates.

Peptide substrates corresponding to p53, histone H3 and histone H4 acetylation sites were selected. Peptides were either unacetylated (H4, H3 and p53), acetylated at one site (e.g. H4K8), or acetylated at more than one site (e.g. H3K9,14). All peptides used are available from Millipore. Recombinant Sirt1 (5 Unit), Sirt2 (0.7 Unit) and Sirt3 (3 Unit) were incubated with indicated acetylated peptide substrates (25 µM) and 1mM β-NAD with the SIRTainty assay. H3K9, H3K9/14, H4K8, and H4K5/8/12/16 displayed high efficiency for deacetylation as compared to the corresponding non-acetylated peptides for all 3 Sirts. Sirt1 and 2 also demonstrated around 2-fold difference in activity with a human p53 peptide (aa 374-389) acetylated at K382 as compared to the non-acetylated peptide, whereas the Sirt3 signal did not differ.

Guidelines for Assay Optimization Using Other Substrates, Test Compounds, and Enzymes

To ensure reliable results using user provided enzymes, substrates and test compounds, it is important to establish appropriate working concentrations for the different components. As appropriate for your assay, perform the different titrations and studies in order to establish specific assay parameter for your reagents. Typical ranges for final concentrations of the various assay components are given in table 3.

- **Sirtuin Titration**

  The optimal sirtuin concentration depends on whether inhibitors or activators are being tested. For inhibitors, a signal of 80~90% of the maximum is recommended, and for activators a signal ~30% of the maximum is recommended. To determine optimal sirtuin concentration, test a range of sirtuin concentrations (0.156-10 units/reaction using a two fold serial dilutions) with a fixed concentration of the acetylated substrate (25 µM) and β-NAD (0.2 mM). Follow the Assay Protocol starting at step 3. Graph the fluorescence observed vs. units of enzyme added. An example of sirtuin titration data is shown in figure 2 on page 10.

- **Kinetic Study**

  Using the optimal sirtuin concentration and conditions established above starting at step 3 of the assay protocol below. Perform a time course experiment to establish the optimal reaction time. Continue the Assay Protocol as indicated omitting the addition of any test compounds. At step 5, the reaction is stopped at different time points by the addition of the detection reagents (0, 5, 10, 15, 30, 60, 90 minutes). Read the fluorescence and graph fluorescence reading for each time point. The optimal reaction time is when the fluorescent signal reaches around 80-90% of the maximum. This optimal reaction time may be used for the remaining experiments.

- **Acetylated Peptide Substrate Titration**

  To determine the K_m for the acetylated peptide substrate, test a range of acetylated peptide substrate (8-200 µM using 5-fold serial dilutions) with the optimized sirtuin concentration and other reagents starting at step 3 of the Assay Protocol. Continue the Assay Protocol as indicated. Graph the fluorescence observed for each substrate concentration. Michaelis-Menten kinetics are applied to acquire the substrate K_m value. Typically, a substrate concentration with less than the K_m value that still gives sufficient fluorescence is chosen when setting up compound screening of sirtuins.

- **β-NAD Titration**

  To determine of the K_m for β-NAD, test a range of β-NAD concentrations (8-1000 µM in a 5-fold serial dilutions) with the optimized sirtuin and acetylated peptide concentrations, and other reagents starting at step 3 of the Assay Protocol. Continue with the Assay Protocol as indicated. Graph the fluorescence observed for each concentration of β-NAD used. Michaelis-Menten kinetics are applied to acquire the K_m value. Typically, a β-NAD concentration with less than the K_m value that still gives sufficient fluorescence is chosen when setting up the compounds screening of sirtuins.

- **Inhibitor compounds IC_{50}**

  The sirtuin activity is tested over a broad range of inhibitor concentrations to generate a dose response curve. The test is generally run using the previously determined optimal assay conditions. A proper β-NAD concentration (reference the K_m value determined at the β-NAD titration step) should be considered. To create the IC_{50} curve, graph the percent inhibition relative to the log of the inhibitor concentration used. An example of IC_{50} data is shown in figure 4 on page 11.

Table 3. Typical final concentration ranges of SIRTainty assay components:

Component	Concentration range
Acetylated peptide substrate	10-50 µM
Sirt1	1.25-5 Units/reaction
β-NAD	50-200 µM
Nicotinamidase	0.2-1 µg/reaction
Developer Reagent	Ready to use

Troubleshooting Guide

Problem	Potential Cause	Experimental Suggestions
No signal or weak signal in all wells	Missing components or key steps	Check to make sure all components were added in the appropriate steps and amounts.
	Recombinant Sirt1 or nicotinamidase is no longer active or has reduced activity	Make sure all components are stored at the recommended temperature and minimize the freeze/thaw cycle as manual recommends. Make aliquots of components when first thawed if planning more than one assay.
	Plate reader or settings are not optimal	Verify the measurement, read time, and filter on the plate reader.
	Incorrect storage temperatures Incorrect assay temperature	Items are to be stored at the appropriate storage temperatures. Performance can be negatively affected if reagents are not stored and used in the appropriate time period.
No detectable signal in test samples	Incomplete Sirt1 enzyme reaction or missing key components.	Check the enzyme reaction setup procedure, and ensure that substrate, β-NAD and nicotinamidase are added to the reaction buffer.
Low Sirt1 activity signal during inhibitor screening	High DMSO concentration or Sirt1 enzyme degraded.	Ensure that the final DMSO concentration in the enzyme reaction does not exceed 2%. Prepare a more concentrated compound stock in DMSO, then further dilute to the desired concentration with SIRTainty™ assay buffer to minimize the DMSO effects.
High background signal with unacetylated peptide	Improper peptide structure for the Sirt reaction.	Screen for a suitable peptide structure that works in the Sirt enzyme reaction.

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