Description

The SIRT1 (Sirtuin1) Fluorogenic Assay Kit is designed to measure SIRT1 (silent mating type information regulation 2 homolog) activity for screening and profiling applications. The assay kit comes in a convenient 96-well format, with enough purified recombinant SIRT1 enzyme (amino acids 193-741), fluorogenic substrate, SIRT Developer and assay buffer for 100 enzyme reactions. This kit also contains the SIRT inhibitor nicotinamide as a control.

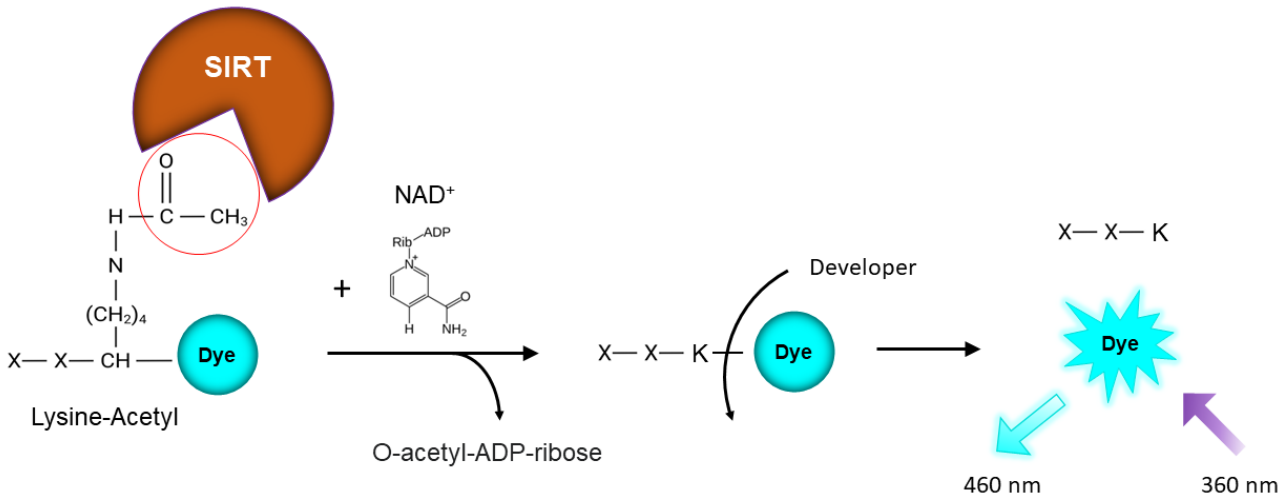

Figure 1: Illustration of the mechanism behind the SIRT1 (Sirtuin1) Fluorogenic Assay Kit.

The fluorescence from dye molecules is quenched when bound to the peptide substrate. SIRT catalyzes the hydrolysis of the acetyl group from the lysine, in a NAD^+-dependent mechanism. Upon incubation with a developer solution specific for non-acetylated lysines, the dye is released and able to fluoresce (λex=350-380 nm; λem=440-460 nm). Fluorescence is thus proportional to SIRT activity.

Background

SIRT1, also known as NAD-dependent deacetylase sirtuin 1 or silent mating type information regulation 2 homolog, is a NAD^+-dependent Class III member of the histone deacetylase family which is involved in lysine deacetylation. Lysine acetylation/deacetylation is a dynamic process involved in the regulation of a variety of cellular functions, similarly to phosphorylation/dephosphorylation. SIRT1 regulates cellular senescence, apoptosis, lipid metabolism, oxidative stress and inflammation. SIRT1 can deacetylate directly several transcription factors and co-factors, such as p53, FOXO1/3/4 (forkhead-box transcription factor 1/3/4), HSF1 (heat shock factor 1), HIF-1α (hypoxia-inducible factor 1 alpha). It can also function indirectly, such as in the case of PPARα/γ (peroxisome proliferator-activated receptor α/γ). SIRT1 is itself regulated by several factors, such as NAD^+/NADH ratio, CCAR2 (cell cycle and apoptosis regulator protein 2), PARP1 (poly ADP-ribose polymerase 1) and PARP2, amongst others. SIRT1 has been linked to inflammation in sepsis, liver, lung and kidney. The development of new molecules specifically targeting SIRT1, in a disease-specific context, and a better understanding of its modes of action may open newer avenues of SIRT1-linked diseases.

Applications

Study enzyme kinetics and screen small molecule activators/inhibitors for drug discovery and high throughput screening (HTS) applications.

Supplied Materials

Catalog #	Name	Amount	Storage
50012-KC50	Sirtuin1 (193-741), GST-Tag*	50 µg	-80°C
50032-KC50	5 mM Fluorogenic HDAC Substrate 1	50 µl	-80°C
50089	2x SIRT Developer (contains 2 mM Nicotinamide)	6 ml	-80°C
78272-KC50	50 mM NAD^+	50 µl	-80°C
83531-KC500	10 mM Nicotinamide	500 µl	-80°C
50090-KC10	SIRT Assay Buffer	10 ml	-20°C
79685	Black, low binding microtiter plate	1	Room Temperature

**The concentration of the protein is lot-specific and will be indicated on the tube.*

Materials Required but Not Supplied

- 1 mg/ml BSA (bovine serum albumin) solution in distilled water
- Fluorimeter capable of excitation at λ=350-380 nm and detection at λ=440-460 nm
- Adjustable micropipettor and sterile tips
- Orbital shaker
- 37°C incubator

Storage Conditions

This assay kit will perform optimally for up to **6 months** from date of receipt when the materials are stored as directed.

Safety

This product is for research purposes only and not for human or therapeutic use. This product should be considered hazardous and is harmful by inhalation, in contact with skin, eyes, clothing, and if swallowed. If contact occurs, wash thoroughly.

Contraindications

- The final concentration of DMSO in the assay should not exceed 1%.
- Compounds that are fluorescent may interfere with the results, depending on their spectral excitation and emission properties.
- It is recommended that the compound alone is tested to determine any potential interference of the compound with the assay results.

Assay Protocol

- All samples and controls should be performed in duplicate.
- The assay should include “Blank”, “Positive Control”, “Control Inhibitor” and “Test Inhibitor” conditions.
- We recommend maintaining the diluted protein on ice during use.

- For detailed information on protein handling please refer to Protein FAQs (bpsbioscience.com).
- We recommend using nicotinamide as internal control. If not running a dose response curve for the control inhibitor, we recommend running the control inhibitor at 0.1X, 1X and 10X the IC_{50} value shown in the validation data below.

1. Thaw **5 mM HDAC Substrate 1, 50 mM NAD^+**, **10 mM Nicotinamide** and **SIRT Assay Buffer.**

2. Prepare the Inhibitor Control by diluting 10 mM Nicotinamide to 1000X the IC_{50} in 100% DMSO. Then dilute 10-fold in SIRT Assay Buffer (the DMSO amount is now 10%) and corresponds to 100X the IC_{50} value (5 µl/ well). Using Diluent Solution prepare solutions at 1X and 10X the IC_{50} value (5 µl/ well).

3. Dilute 50-fold the 5 mM HDAC Substrate 1 with SIRT Assay Buffer (5 µl/well will be needed). This makes **100 µM HDAC Substrate 1**.

4. Thaw **SIRT1** on ice. Briefly spin the tube to recover the full content.

5. Dilute SIRT1 to 100 ng/µl (5 µl/well) with SIRT Assay Buffer.

6. Prepare a **Master Mix** (35 µl/well): N wells x (29.5 µl of SIRT Assay Buffer + 5 µl of 1 mg/ml BSA + 0.5 µl of 50 mM NAD^+).

7. Add 35 µl of Master Mix to every well.

8. Prepare the **Test Inhibitor** (5 µl/well): for a titration, prepare serial dilutions at concentrations 10-fold higher than the desired final concentrations. The final volume of the reaction is 50 µl.

   8.1 If the Test Inhibitor is water-soluble, prepare 10-fold more concentrated serial dilutions of the inhibitor than the desired final concentrations in SIRT Assay Buffer.

   For the positive and negative controls, use SIRT Assay Buffer (Diluent Solution).

   OR

   8.2 If the Test inhibitor is soluble in DMSO, prepare the test inhibitor at a concentration 100-fold higher than the highest desired concentration in 100% DMSO, then dilute the inhibitor 10-fold in SIRT Assay Buffer to prepare the highest concentration of the 10-fold intermediate dilutions. The concentration of DMSO is now 10%.

   Using SIRT Assay Buffer containing 10% DMSO to keep the concentration of DMSO constant, prepare serial dilutions of the Test Inhibitor at 10-fold the desired final concentrations.

   For positive and negative controls, prepare 10% DMSO in SIRT Assay Buffer (vol/vol) so that all wells contain the same amount of DMSO (Diluent Solution).

   Note: The final concentration of DMSO should not exceed 1%.

9. Add 5 µl of Test Inhibitor to each well labeled “Test Inhibitor”.

10. Add 5 µl of Diluent Solution to the “Positive Control" and “Blank” wells.

11. Add 5 µl of diluted Nicotinamide to the “Control Inhibitor” wells.

12. Add 5 µl of SIRT Assay Buffer to the wells designated as "Blank".

13. Add 5 µl of diluted SIRT1 to the wells designated “Positive Control”, “Control Inhibitor” and "Test Inhibitor".

14. Incubate at 37°C for 30 minutes.

15. Initiate the reaction by adding 5 µl of diluted HDAC Substrate 1 (100 µM).

16. Incubate at 37°C for 30 minutes.

Component	**Blank**	**Positive Control**	**Control Inhibitor**	**Test Inhibitor**
Master Mix	35 µl	35 µl	35 µl	35 µl
Test Inhibitor	-	-	-	5 µl
Diluted Nicotinamide	-	-	5 µl	-
Diluent Solution	5 µl	5 µl	-	-
SIRT Assay Buffer	5 µl	-	-	-
Diluted SIRT1 (100 ng/µl)	-	5 µl	5 µl	5 µl
Diluted HDAC Substrate 1 (100 µM)	5 µl	5 µl	5 µl	5 µl
Total	**50 µl**	**50 µl**	**50 µl**	**50 µl**

17. Add 50 µl of **2x SIRT Developer** to each well.

18. Incubate at Room Temperature for 15 minutes.

19. Immediately read in a fluorimeter or a microplate reader capable of excitation at λ=350-380 nm and detection at λ=440-460 nm.

20. The “Blank” value is subtracted from all other readings.

Example Results

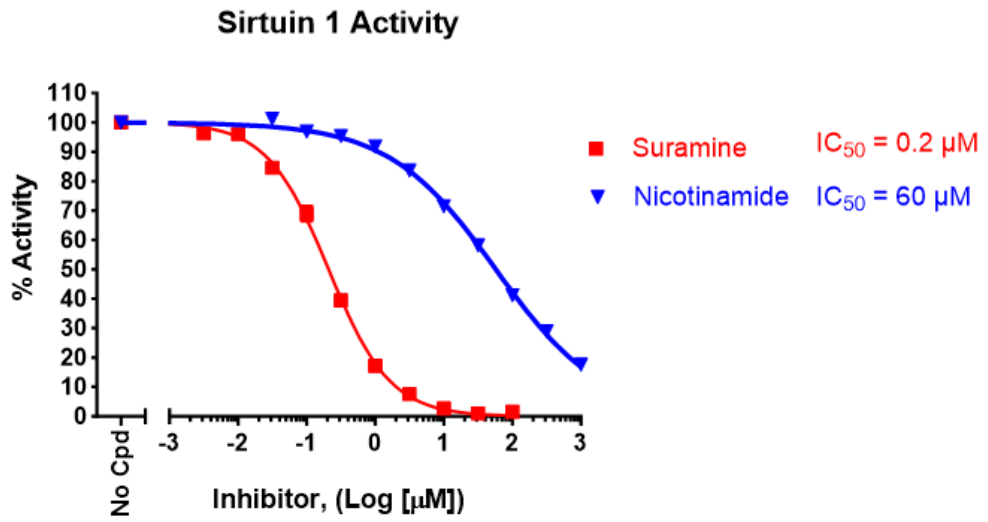

Figure 2: Inhibition of SIRT1 activity by the inhibitors Suramin and Nicotinamide.
SIRT1 activity was measured in the presence of increasing concentrations of Suramin (Cayman Chemicals #11126) and Nicotinamide. The "Blank" value was subtracted from all other values. Results are expressed as the percent of control (activity in the absence of inhibitor, set at 100%).

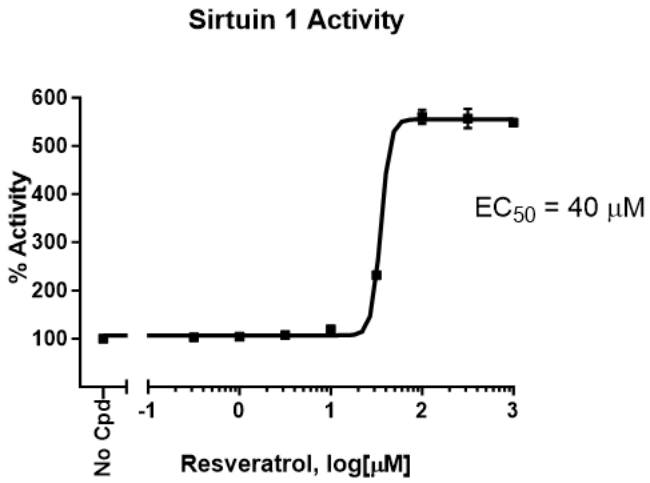

Figure 3: Activation of SIRT1 activity by Reverastrol.
SIRT1 activity was measured in the presence of increasing concentrations of Reverastrol. The "Blank" value was subtracted from all other values. Results are expressed as the percent of control (activity in the absence of inhibitor, set at 100%).

Data shown is representative.

Troubleshooting Guide

Visit bpsbioscience.com/assay-kits-faq for detailed troubleshooting instructions**.** For lot-specific information and all other questions, please visit https://bpsbioscience.com/contact.

References

Barlev N., *et al.*, 2001 *Mol Cell* 8:1243.
Yang Y., *et al.*, 2022 *Front Immunol* 13:831168.

Related Products

Products	*Catalog #*	*Size*
Sirtuin 2, His-Tag Recombinant	50013	100 µg
Sirtuin 3, GST-Tag Recombinant	50014	100 µg
Sirtuin 4, GST-Tag Recombinant	50015	100 µg
SIRT2 (Sirtuin2) Fluorogenic Assay Kit	50087	96 reactions
SIRT3 (Sirtuin3) Fluorogenic Assay Kit	50088	96 reactions
SIRT5 (Sirtuin5) Fluorogenic Assay Kit	50085	96 reactions

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