3050 Spruce Street, St. Louis, MO 63103 USA
Tel: (800) 521-8956 (314) 771-5765 Fax: (800) 325-5052 (314) 771-5757
email: techservice@sial.com sigma-aldrich.com

Product Information

SIRT1 Assay Kit

Product Number **CS1040**
Storage Temperature –20 °C

TECHNICAL BULLETIN

Product Description

Sirtuins (Sir2) are an evolutionarily conserved family of NAD^+ dependent histone/protein deacetylases that tightly couple the cleavage of NAD^+ and the deacetylation of protein substrates. The reaction products are nicotinamide, the deacetylated product, and a novel metabolite, 2′-O-acetyl-ADP-ribose.[1,2] The proteins within this family are named after the first protein discovered from this family, Sir2 (**S**ilent **I**nformation **R**egulator 2). Besides gene silencing, sirtuin proteins are important in other processes such as cell cycling regulation[3] and fatty acid metabolism.[4] SIRT1 is the human homolog of Sir2 and the one most studied to date. It mediates p53 dependent process,[5] transcription regulation, muscle differentiation, adipogenesis, and protection from axonal degeneration.[1] SIRT1 also participates in early embryogenesis, neurogenesis, and cardiogenesis.[6]

The assay procedure is based on a two-step enzymatic reaction. The first step is deacetylation by SIRT1 of a substrate that contains an acetylated lysine side chain. The second step is the cleavage of the deacetylated substrate by the Developing Solution and the release of a highly fluorescent group. The measured fluorescence is directly proportional to the deacetylation activity of the enzyme in the sample.

The kit offers all the reagents required for the fast and easy measurement of purified SIRT1 activity and for screening of inhibitors/activators. Moreover, the kit contains an inhibitor (nicotinamide) and an activator (resveratrol) as negative and positive controls, respectively.

Components

The kit contains sufficient reagents for 100 assays of 50 μL reaction volume in a 96 well plate format.

Assay Buffer Product Number A6480	20 mL
SIRT1 Substrate (Fluorometric) Product Number S9821	100 μL
Standard (non-acetylated) 20 mM Product Number S9946	100 μL
Developing Solution Product Number D5068	1.5 mL
Nicotinamide Solution (inhibitor) Product Number N1788	100 μL
SIRT1 human, recombinant expressed in *E. coli* Product Number S8446	150 μg
NAD^+ Solution Product Number N1663	1 mL
Resveratrol Solution (activator) Product Number R0530	100 μL

Equipment and Reagents Required but Not Provided

- Corning® half-area 96 well plates, white polystyrene, nonbonding surface (Product Number CLS3992)
- Fluorometer

Precautions and Disclaimer

This product is for R&D use only, not for drug, household, or other uses. Please consult the Material Safety Data Sheet for information regarding hazards and safe handling practices.

Preparation Instructions

Inhibitor Solution – Thaw the Nicotinamide Solution (Product Number N1788) and mix until homogenous. Dilute an aliquot of the Nicotinamide Solution 50-fold in Assay Buffer (Product Number A6480). Store the remaining Nicotinamide Solution at –20 °C. 5 μL of the diluted Inhibitor Solution are sufficient for a single reaction (a single well).

SIRT1 Substrate Solution – Thaw the SIRT1 Substrate (Fluorometric), Product Number S9821, at room temperature (it will freeze on ice) and mix until homogenous. Dilute an aliquot of the SIRT1 Substrate (Fluorometric) 16-fold with Assay Buffer (Product Number A6480). Store the remaining SIRT1 Substrate (Fluorometric) at –20 °C. 10 μL of the diluted SIRT1 Substrate Solution are sufficient for a single reaction (a single well).

Activator Solution - Thaw the Resveratrol Solution (Product Number R0530) at room temperature (it will freeze on ice) and mix until homogenous. Dilute an aliquot of the Resveratrol Solution 100-fold in Assay Buffer (Product Number A6480). Store the remaining Resveratrol Solution at –20 °C. 5 μL of the diluted Activator Solution are sufficient for a single reaction (a single well).

400 μM Standard Solution - Thaw the Standard (non-acetylated), Product Number S9946, at room temperature and mix until homogenous. Dilute an aliquot of the Standard (non-acetylated) 50-fold with Assay Buffer (Product Number A6480) to obtain a 400 μM Standard Solution. Store the remaining Standard (non-acetylated) at –20 °C. 30 μL of the 400 μM Standard Solution are sufficient for a single standard curve.

SIRT1 - Use ~1.5 μg of SIRT1 (Product Number S8446) enzyme per reaction. The concentration of the enzyme is indicated on the CoA for the lot.

Storage/Stability
The kit is shipped on dry ice and storage at –20 °C is recommended. The Assay Buffer (Product Number A6480) can be stored at 2–8 °C.

Procedure
The assay for SIRT1 activity and the determination of the standard curve are described as separate procedures. As an option, both procedures can be performed in parallel on the same plate. In this case the standard samples can be incubated with the reaction samples, before the addition of the Developing Solution.

Keep on ice the prepared solutions (SIRT1 Substrate Solution, Inhibitor Solution, and Activator Solution) and the thawed homogenous kit components (Developing Solution [Product Number D5068], SIRT1 [Product Number S8446], and the NAD^+ Solution [Product Number N1663]).

Set the fluorometer at the appropriate sensitivity and wavelengths:

Excitation = 340-380 nm
Emission = 430-460 nm

Optimize the wavelengths according to the instrument used.

Perform the assay in duplicates. The assay described is for a 50 μL final reaction volume. If the device used dictates a 100 μL final reaction volume, multiply by 2 the amount of reagents used for the reaction.

A. Standard Curve
The standard curve, as described, should be performed in two sets. The first set is intended to measure the fluorescence at different concentrations of the standard and the second set, without Developing Solution, is a standard blank.

Table 1.
Standard Curve Scheme

Well No.	Assay buffer	Standard Solution (400 μM)	Standard amount/ well	Standard final conc.
1, 2	47.5 μL	2.5 μL	1 nmole	20 μM
3, 4	45 μL	5 μL	2 nmole	40 μM
5, 6	42.5 μL	7.5 μL	3 nmole	60 μM
7, 8	40 μL	10 μL	4 nmole	80 μM

1. Prepare two sets of the standard curve in a 96 well plate according to Table 1.
   Note: At this stage you can also set up the test samples on the same plate, according to steps B1–4 (including 30 minute incubation of the plate).
2. Add 5 μL of Developing Solution to each well in one set of the standard dilution series. To the second set, add 5 μL of Assay Buffer instead of the Developing Solution to each well. Mix using a horizontal shaker or by pipetting.
3. Incubate the plate at 37 °C for 10 minutes.
4. Read the fluorescence in a plate reader.
5. Determine the net fluorescence signal of each standard sample by subtracting the fluorescence signal of the parallel control sample (without Developing Solution).
6. Plot the fluorescence signal (y-axis) versus concentration of the Standard (x-axis). Determine the slope as FU/μM.

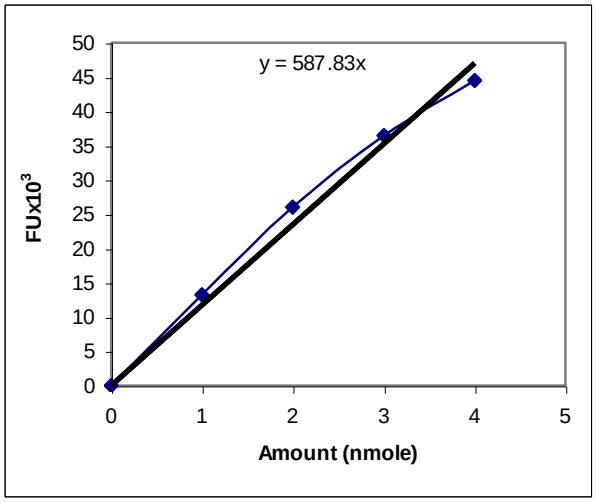
B. Assay for SIRT1 activity (in the presence or absence of an inhibitor/activator)

Table 2.
Reaction Scheme for SIRT1 Activity or Activator/Inhibitor Screening

Well No.	Assay	Assay Buffer (μL)	SIRT1 (~1.5 μg)	NAD^+ Solution (μL)	Inhibitor Solution (μL)	Activator Solution (μL)
1, 2	SIRT1 purified enzyme	35–x	x μL	5	–	–
3, 4	Inhibition reaction	30–x	x μL	5	5	–
5, 6	Activation reaction	30–x	x μL	5	–	5
7, 8	Blank	40–x	x μL	–	–	–

1. Set up the assay samples according to Table 2.
   Notes:
   - The assay blank is the reaction without NAD^+.
   - The amount of enzyme used in the assay is ~1.5 μg. Calculate the volume of enzyme to be used for the assay accordingly. A lower amount can be used down to a minimum of 1 μg.
2. Add 10 μL of the SIRT1 Substrate Solution to each well.
3. Mix for several seconds using a horizontal shaker.
4. Incubate the plate at 37 °C for 30 minutes.
5. Add 5 μL of the Developing Solution to each well. Mix using a horizontal shaker or by pipetting, and incubate at 37 °C for 10 minutes.
6. Read the fluorescence in the plate reader.
7. Determine the net fluorescence signal of the sample(s) by subtracting the fluorescence signal of the blank.
8. Calculate the sample activity using the standard curve.

Results
Figure 1.
A Typical Standard Curve



This curve is for illustration only. A standard curve must be determined by each user.

References

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