SIRT1 Assay Kit
Catalog Number CS1040
Storage Temperature –20 °C

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. The proteins within this family are named after the first protein discovered from this family, Sir2 (Silent Information Regulator 2). Besides gene silencing, sirtuin proteins are important in other processes such as cell cycling regulation and fatty acid metabolism. SIRT1 is the human homolog of Sir2 and the one most studied to date. It mediates p53 dependent processes, transcription regulation, muscle differentiation, adipogenesis, and protection from axonal degeneration. SIRT1 also participates in early embryogenesis, neurogenesis, and cardiogenesis.

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: 20 ml
  - Catalog Number A6480
- SIRT1 Substrate (Fluorometric): 100 µl
  - Catalog Number S9821
- Standard (non-acetylated) 20 mM: 100 µl
  - Catalog Number S9946
- Developing Solution: 1.5 ml
  - Catalog Number D5068
- Nicotinamide Solution (inhibitor): 100 µl
  - Catalog Number N1788
- SIRT1: 150 µg human, recombinant expressed in E. coli
  - Catalog Number S8446
- NAD* Solution: 1 ml
  - Catalog Number N1663
- Resveratrol Solution (activator): 100 µl
  - Catalog Number R0530

Equipment and Reagents Required but Not Provided
- Corning® half-area 96 well plates, white polystyrene, nonbonding surface (Catalog 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 (Catalog Number N1788) and mix until homogenous. Dilute an aliquot of the Nicotinamide Solution 50-fold in Assay Buffer (Catalog 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), Catalog 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 (Catalog 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 (Catalog 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 (Catalog 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), Catalog Number S9946, at room temperature and mix until homogenous. Dilute an aliquot of the Standard (non-acetylated) 50-fold with Assay Buffer (Catalog 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 (Catalog Number S8446) enzyme per reaction. The concentration of the enzyme is indicated on the label.

Storage/Stability
The kit is shipped on dry ice and storage at –20 °C is recommended. The Assay Buffer (Catalog 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 [Catalog Number D5068], SIRT1 [Catalog Number S8446], and the NAD⁺ Solution [Catalog 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

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>1, 2</td>
<td>47.5 µl</td>
<td>2.5 µl</td>
<td>1 nmole</td>
<td>20 µM</td>
</tr>
<tr>
<td>3, 4</td>
<td>45 µl</td>
<td>5 µl</td>
<td>2 nmole</td>
<td>40 µM</td>
</tr>
<tr>
<td>5, 6</td>
<td>42.5 µl</td>
<td>7.5 µl</td>
<td>3 nmole</td>
<td>60 µM</td>
</tr>
<tr>
<td>7, 8</td>
<td>40 µl</td>
<td>10 µl</td>
<td>4 nmole</td>
<td>80 µM</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Well No.</th>
<th>Assay</th>
<th>Assay Buffer (µl)</th>
<th>SIRT1 (~1.5 µg)</th>
<th>NAD⁺ Solution (µl)</th>
<th>Inhibitor Solution (µl)</th>
<th>Activator Solution (µl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1, 2</td>
<td>SIRT1 purified enzyme</td>
<td>35–x</td>
<td>x µl</td>
<td>5</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>3, 4</td>
<td>Inhibition reaction</td>
<td>30–x</td>
<td>x µl</td>
<td>5</td>
<td>5</td>
<td>–</td>
</tr>
<tr>
<td>5, 6</td>
<td>Activation reaction</td>
<td>30–x</td>
<td>x µl</td>
<td>5</td>
<td>–</td>
<td>5</td>
</tr>
<tr>
<td>7, 8</td>
<td>Blank</td>
<td>40–x</td>
<td>x µl</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
</tbody>
</table>

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

![A Typical Standard Curve](image)

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


Corning is a registered trademark of Corning, Inc.