Gentaur Europe BVBA Voortstraat 49, 1910 Kampenhout BELGIUM
Tel 0032 16 58 90 45 info@gentaur.com

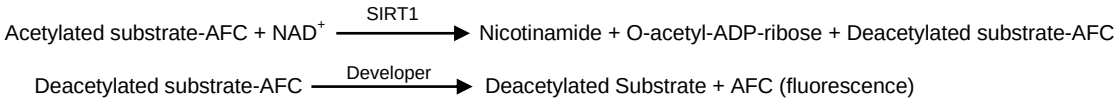
# SIRT1 Inhibitor/Activator Screening Kit (Fluorometric)

12/15

**(Catalog # K325-100; 100 assays; Store kit at -80 °C)**

## I. Introduction:

SIRT1 or Sirtuin 1 is a member of the silent information regulator 2 family. SIRT1, an NAD-dependent histone deacetylase can deacetylate histones and a number of nonhistone substrates, including p53. It is predominantly localized in the nucleus of normal cells. In cancer cells however, it is predominantly localized in the cytoplasm. SIRT1 has been shown to regulate a wide range of cellular functions that affect metabolic homeostasis and aging. SIRT1 exerts anti-apoptotic, anti-oxidative, and anti-inflammatory effects against cellular injury, and protects the cells through the regulation of mitochondrial biogenesis, autophagy, and metabolism in response to the cellular energy and redox status. SIRT1 also promotes vasodilation and protects vascular tissues. Activation and inhibition of SIRT1 is being targeted for various diseases. Unlike other known protein deacetylases, which simply hydrolyze acetyl-lysine residues, the sirtuin-mediated deacetylation reaction hydrolyzes acetyl-lysine and NAD. This hydrolysis yields the deacetylated substrate, O-acetyl-ADP-ribose and nicotinamide, itself an inhibitor of sirtuin activity. In BioVision's SIRT1 Inhibitor/Activator screening Kit, Sirtuin 1 deacetylates the substrate, followed by cleavage of the deacetylated substrate to release the fluorescent group, which is detected fluorometrically at Ex/Em = 400/505 nm. In the presence of SIRT1 inhibitor, deacetylation is impeded, preventing cleavage of the substrate and release of the fluorescent group. The SIRT1 activator enhances SIRT1 activity resulting in a higher fluorescent signal in comparison to the control. This kit provides a rapid, simple, sensitive, and reliable test, which is suitable for high-throughput screening of SIRT1 inhibitors/activators. Inhibitor control (Nicotinamide) is included to compare the efficacy of the test inhibitors.




## II. Application:

- Screening/characterizing/studying SIRT1 inhibitors and activators

## III. Kit Contents:

| Components | K325-100 | Cap Code | Part Number |
|---|---|---|---|
| SIRT1 Assay Buffer | 25 ml | WM | K325-100-1 |
| 1 M DTT | 0.4 ml | Green | K325-100-2 |
| Substrate (in DMSO) | 0.2 ml | Red | K325-100-3 |
| NAD | 1 vial | Purple | K325-100-4 |
| SIRT1 Enzyme | 0.2 ml | Brown | K325-100-5 |
| Inhibitor (Nicotinamide, 4 mM) | 0.9 ml | Blue | K325-100-6 |
| Developer | 1 ml | Orange | K325-100-7 |

## IV. User Supplied Reagents and Equipments:

- 96-well plate with flat bottom. White plates are preferred for this assay.
- Fluorescence microplate reader

## V. Storage Condition and Reagent Preparation:

Store kit at -80°C, protected from light. Avoid repeated freeze/thaw for all non-buffer components. Briefly centrifuge small vials before opening. Read entire protocol before performing the assay.

- **SIRT1 Assay Buffer:** Store at 4°C or -20°C. Warm to 37°C and add DTT to final concentration of 2 mM just before use. Make fresh as needed.
- **1 M DTT:** Store at -20°C. Thaw and keep on ice while in use. Use within two months.
- **Substrate:** Aliquot and Store at -20°C. Avoid repeated freeze/thaw. Use fresh tip each time. Use within two months.
- **NAD:** Reconstitute with 220 µl deionized water. Aliquot and store at -80°C. Avoid repeated freeze/thaw. Before use, dilute NAD by adding 2 µl of NAD stock solution to 58 µl of SIRT1 Assay Buffer without DTT. Make as much as needed. Use within two months.
- **SIRT1 Enzyme:** Thaw and mix gently by pipetting. Aliquot and store at -80°C. Use within two months.
- **Inhibitor (Nicotinamide):** Store at -20°C. Keep on ice while in use.
- **Developer:** Aliquot and store at -20°C. Avoid repeated freeze/thaw. Keep on ice while in use.

## VI. SIRT1 Inhibitor/Activator Screening Protocol:

1. **Enzyme Solution Preparation:** Mix enough reagents for the number of assays to be performed. For each well, prepare 25 µl SIRT1 Enzyme Solution:

| | |
|---|---|
| SIRT1 Assay Buffer (with DTT) | 23 µl |
| SIRT1 Enzyme | 2 µl |

   Mix & add 25 µl of the SIRT1 Enzyme Solution into desired wells.

2. **Screen compounds, Inhibitor Control, Enzyme Control & Blank Control Preparations:** Dissolve candidate inhibitors/activators at 1000X highest final test concentration into an appropriate solvent. Dilute to 4X the desired test concentration with SIRT1 Assay Buffer (with DTT). Add 25 µl Inhibitor (Nicotinamide), Assay Buffer or diluted test inhibitor/activator into SIRT1 Enzyme solution wells as Inhibitor Control, Enzyme Control [EC] (no inhibitor) or sample screen [S]. Add 50 µl SIRT1 Assay Buffer into one well as Blank Control (no enzyme). Mix well, and incubate the plate for 5 min. at 37°C.

**Note:** High solvent concentration might affect the enzymatic activity. Prepare parallel well(s) as solvent control to test the effect of the solvent on enzyme activity.

3. **Substrate preparation:** For each well, prepare 40 µl of Substrate solution.

| | |
|---|---|
| SIRT1 Assay Buffer (with DTT) | 36 µl |
| Substrate | 2 µl * |
| Diluted NAD | 2 µl |

Add 40 µl of the Substrate solution into each well. Mix & incubate at 37°C for 30-60 min.
*** NOTE:** To screen for Activators, dilute the Substrate 10x in DMSO and use 2 µl to prepare the Substrate Solution.

4. **Develop:** Add 10 µl Developer into each well. Mix well and incubate for 10 min. at 37°C, protected from light.
5. **Measurement:** Read fluorescence (Ex/Em = 400/505 nm).
6. **Calculation:** Subtract the Blank Control reading from all readings to obtain ΔRFU for each reading. Set the ΔRFU of Enzyme Control [EC] as 100%, and calculate % Inhibition or % Relative Activity of the test inhibitors or % Activation of activators as follows:

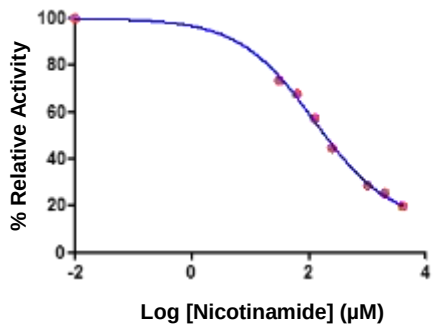
$$\% \text{ Inhibition} = \frac{\Delta\text{RFU of EC} - \Delta\text{RFU of S}}{\Delta\text{RFU of EC}} \times 100$$

$$\% \text{ Relative Activity} = \frac{\Delta\text{RFU of S}}{\Delta\text{RFU of EC}} \times 100$$

$$\% \text{ Activation} = \frac{\Delta\text{RFU of S} - \Delta\text{RFU EC}}{\Delta\text{RFU of EC}} \times 100$$

$$\text{Fold Activation} = \frac{\Delta\text{RFU of S}}{\Delta\text{RFU of EC}} \times 100$$

(a)




(b)

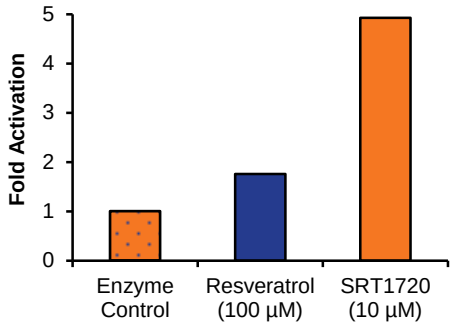



**Figure:** (a) Inhibition of SIRT1 Enzyme activity by Nicotinamide. $IC_{50}$ of Nicotinamide for SIRT1 was determined to be 124.2 µM. (b) Activation of SIRT1 using 100 µM Resveratrol and 10 µM SRT1720. Resveratrol and SRT1720 increase SIRT1 Activity by 1.5 fold and 5 fold respectively. Assays are performed using the kit protocol.

## VII. Related Products:

Sirtuin 1 (human intracellular) ELISA Kit (K4923)
Sirtuin Activity Assay Kit (Fluorometric) (K324)
SIRT2 Inhibitor Screening Assay Kit (Fluorometric) (K322)
HDAC Colorimetric Activity Assay Kit (K331)
Sirtuin 2, human recombinant (7632)
SIRT5 (GST-tagged), Human recombinant (7674)
SIRT4 (GST-tagged), Human recombinant (7673)
Sirtuin 6, human recombinant (7578)
Sirtinol (2062)
SRT1720 (2772)
Sirtuin 2 (human intracellular) ELISA Kit (K4924)
SIRT6 Inhibitor Screening Kit (Fluorometric) (K323)
HDAC Fluorometric Activity Assay Kit (K330)
*InSitu* HDAC Activity Fluorometric Assay Kit (K339)
Active SIRT6 (GST-tagged), human recombinant (7697)
Active SIRT6 (His-tagged), human recombinant (7699)
SIRT1 (193-747 aa) (GST-tagged) (7264)
SIRT7 (2-400 aa) (His-tagged), Human recombinant (7675)
Resveratrol (1758)

***FOR RESEARCH USE ONLY! Not to be used on humans.***