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Product Information

SIRT2 Inhibitor Screening Assay Kit

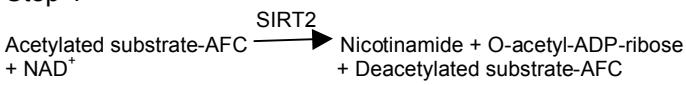
Catalog Number EPI010
Storage Temperature – 20 °C

TECHNICAL BULLETIN

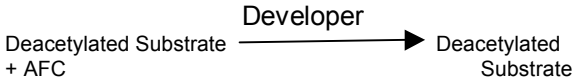
Product Description
SIRT2 is a member of the Sirtuin class of proteins, which are enzymes with either histone deacetylase or mono-ribosyltransferase activity. Sirtuins have been shown to influence transcription, apoptosis, aging, and stress resistance, as well as energy efficiency and alertness in animals on low-calorie diets. Unlike other known deacetylases, which only hydrolyze acetyl-lysine residues, the sirtuins couple lysine deacetylation to NAD hydrolysis. This hydrolysis yields O-acetyl-ADP-ribose, the deacetylated substrate and nicotinamide, the latter of which is an inhibitor of sirtuin activity. Studies indicate that human sirtuins may function as intracellular regulatory proteins with mono-ADP-ribosyltransferase activity.

With Sigma's Sirtuin Inhibitor Screening Kit, Sirtuin deacetylates the Substrate, and then a Developer cleaves the deacetylated substrate to release a fluorescent group, the latter of which can be detected at Ex/Em = 395/541 nm. In the presence of a SIRT inhibitor, the deacetylation will be impeded, preventing substrate cleavage and release of the fluorescent group. The kit provides a rapid, simple, sensitive, and reliable test, which is suitable for both low and high-throughput screening of SIRT2 inhibitors. A positive control inhibitor, Nicotinamide, is included to compare with the efficacy of the test inhibitors.

Step 1



Step 2



Components
The kit is sufficient for 100 assays in 96 well plates.

Assay Buffer (WM cap) Catalog Number EPI010A	25 mL
Substrate (Red cap) Catalog Number EPI010B	0.2 mL
Cofactor (Purple cap) Catalog Number EPI010C	1 vl
Developer (Orange cap) Catalog Number EPI010D	1 ml
SIRT2 Enzyme (Green cap) Catalog Number EPI010E	0.5 ml
Inhibitor Control (Nicotinamide)(Blue cap) Catalog Number EPI010F	1 mL

Reagents and equipment required but not provided.
96 well flat-bottom plate – It is recommended to use a black plate with flat clear bottom.
Fluorometric multiwell plate reader (ELISA reader).

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.

Storage/Stability
The kit is shipped on wet ice. All components should be stored at –20 °C, protected from light.

Preparation Instructions
Briefly centrifuge vials before opening. Use ultrapure water for the preparation of reagents. To maintain reagent integrity, avoid repeated freeze/thaw cycles. Read the entire protocol before performing the assay.

Assay Buffer (EPI010A)
Store at –20°C. Allow Assay Buffer to warm to room temperature before use.

Cofactor (EPI010C) Reconstitute with 220 µL ddH2O. Aliquot and Store at –20°C. Avoid repeated freeze/thaw cycles. Use within one month

Procedure
All samples and standards should be run in duplicate.

1. Add 5 µL SIRT2 Enzyme to each well.

2. Preparation for Inhibitors to be screened: inhibitor controls and blank controls.

a. Dissolve inhibitors to be screened into their proper solvent.
b. Dilute each inhibitor sample with Assay Buffer to 2X the desired final test concentration. Inhibitor Control (Nicotinamide) already contains Assay Buffer.
c. Add 45 µL diluted test inhibitors [S], Inhibitor Control (Nicotinamide) or Assay Buffer alone (Enzyme Control [EC]) into SIRT2 enzyme wells. Be sure to include a solvent only negative control reaction to verify that the solvent itself does not inhibit SIRT2.
d. Mix well, and incubate for 5 minutes at 37 °C.

3.. Substrate preparation

a. For each well, prepare 40 µL of substrate solution.
   36 µL Assay Buffer
   2 µL Substrate
   2 µL Cofactor
b. Mix, add 40 µL of the substrate solution into each well.
c. Mix, incubate at 37 °C for 60 minutes

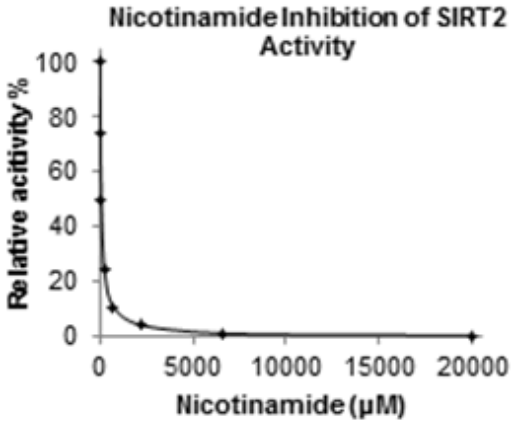
4 Measurement
Read fluorescence (R_0) at Ex/Em = 395/541 nm.

5. Develop:
a. Add 10 µL Developer to each well
b. Mix well and incubate for 10 minutes at 37 °C, protected from light.
c. Read again fluorescence (R_1) at Ex/Em = 395/541 nm

Results
Calculations

The RFU of fluorescence generated by hydrolyzation of substrate is $\Delta RFU = R_1 - R_0$. Set the ΔRFU of Enzyme Control [EC] as 100%, and calculate the relative % inhibition of the test inhibitors [S] as

% Inhibition = [(ΔRFU EC – ΔRFU S)/ ΔRFU EC] x 100%



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