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Product Information

SIRT6 Inhibitor Screening Kit (Fluorometric)

Catalog Number **EPI017**
Storage Temperature –20 °C

TECHNICAL BULLETIN

Product Description

SIRT6 or Sirtuin 6 proteins are a class of proteins that possess either histone deacetylase or monoribosyl-transferase activity. SIRT6 is a nuclear sirtuin that has been associated with aging, cellular protection, sugar metabolism, and certain types of cancer. Broad therapeutic applications are foreseen for SIRT6 inhibitors, including uses in diabetes, immune-mediated disorders, and cancer.

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.

Studies suggest the human sirtuins may function as intracellular regulatory proteins with mono-ADP-ribosyltransferase activity. In this Sirtuin 6 inhibitor screening kit, Sirtuin 6 deacetylates the substrate, followed by cleavage of the deacetylated substrate to release the fluorescent group, which is detected fluorometrically (λ_{ex} = 400 nm/λ_{em} = 505 nm).

In the presence of a SIRT inhibitor, deacetylation is impeded, preventing cleavage of the substrate and release of the fluorescent group. This kit provides a rapid, simple, sensitive, and reliable test, which is suitable for high-throughput screening of SIRT6 inhibitors. Inhibitor control (Nicotinamide) is included to compare the efficacy of the test inhibitors.

Components

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

SIRT6 Assay Buffer Catalog Number EPI017A	25 mL
1 M DTT Catalog Number EPI017B	0.4 mL
Substrate (DMSO) Catalog Number EPI017C	0.2 mL
Cofactor Catalog Number EPI017D	1 vial
Developer Catalog Number EPI017E	1 mL
SIRT6 Enzyme Catalog Number EPI017F	100 µL
Inhibitor (Nicotinamide, 4 mM) Catalog Number EPI017G	0.9 mL

Reagents and Equipment Required but Not Provided.

- 96 well flat-bottom plate – Black plates are preferred for this assay.
- Fluorescence multiwell plate reader

Precautions and Disclaimer

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

Preparation Instructions

Avoid repeated freeze/thaw cycles for all non-buffer components. Briefly centrifuge small vials at low speed prior to opening. Use ultrapure water for the preparation of reagents.

SIRT6 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 – Once thawed, aliquot and store at –70 °C.

Cofactor – Reconstitute with 220 µL of water. Aliquot and store at –70 °C. Avoid repeated freeze/thaw cycles. Use within two months.

Developer – Store at –70 °C once thawed. Keep on ice while in use.

SIRT6 Enzyme – Thaw and mix gently by pipetting. Aliquot and store at –20 °C. Use within two months.

Storage/Stability
Store kit at –20 °C, protected from light. Briefly centrifuge small vials prior to opening.

Procedure
Read entire protocol before performing the assay.

Enzyme Solution Preparation
Mix enough reagents for the number of assays to be performed. For each well, prepare 25 µL of SIRT6 Enzyme Solution, see Table 1.

Table 1.
Preparation of Enzyme Solution

Reagents	Volume
AHCY Assay Buffer	24 µL
AHCY Enzyme	1 µL

Mix and add 25 µL of the SIRT6 Enzyme Solution into desired wells.

Screen compounds, Inhibitor Control, Enzyme Control, and Blank Control Preparations
Dissolve candidate inhibitors at 1,000× highest final test concentration into an appropriate solvent. Dilute to 4× the desired test concentration with SIRT6 Assay Buffer.

Add 25 µL of Inhibitor, Assay Buffer, or diluted test inhibitor into SIRT6 Enzyme solution wells as Inhibitor Control (Nicotinamide), Enzyme Control [EC] (no inhibitor), or sample screen [S].

Mix well and incubate for 5 minutes at 37 °C. Add 50 µL of SIRT6 Assay Buffer into one well as Blank Control.

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.

Substrate preparation
Dilute Cofactor by adding 2 µL of Cofactor solution to 58 µL of SIRT6 Assay Buffer (without DTT) just before use. Make as much as needed. For each well, prepare 40 µL of Substrate solution, see Table 2.

Table 2.
Preparation of Substrate

Reagents	Reaction Mix
SIRT6 Assay Buffer	36 µL
Substrate	2 µL
Diluted Cofactor	2 µL

Add 40 µL of the Substrate solution into each well. Mix and incubate at 37 °C for 60 minutes.

Develop
Add 10 µL of Developer into each well. Mix well and incubate for 10 minutes at 37 °C, protected from light.

Measurement
Read fluorescence (λ_{ex} = 400 nm/λ_{em} = 505 nm).

Results
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 as:

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

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

Troubleshooting Guide

Problem	Possible Cause	Suggested Solution
Assay Not Working	Cold assay buffer	Assay Buffer must be at room temperature
	Omission of step in procedure	Refer and follow Technical Bulletin precisely
	Plate reader at incorrect wavelength	Check filter settings of instrument
	Type of 96 well plate used	Black plates are recommended for this assay
Samples with erratic readings	Samples prepared in different buffer	Use the Assay Buffer provided or refer to Technical Bulletin for instructions
	Cell/Tissue culture samples were incompletely homogenized	Repeat the sample homogenization, increasing the length and extent of homogenization step.
	Samples used after multiple freeze-thaw cycles	Aliquot and freeze samples if samples will be used multiple times
	Presence of interfering substance in the sample	If possible, dilute sample further
	Use of old or inappropriately stored samples	Use fresh samples and store correctly until use
Lower/higher readings in samples and standards	Improperly thawed components	Thaw all components completely and mix gently before use
	Use of expired kit or improperly stored reagents	Check the expiration date and store the components appropriately
	Allowing the reagents to sit for extended times on ice	Prepare fresh Reaction Mix before each use
	Incorrect incubation times or temperatures	Refer to Technical Bulletin and verify correct incubation times and temperatures
	Incorrect volumes used	Use calibrated pipettes and aliquot correctly
Non-linear standard curve	Use of partially thawed components	Thaw and resuspend all components before preparing the reaction mix
	Pipetting errors in preparation of standards	Avoid pipetting small volumes
	Pipetting errors in the Reaction Mix	Prepare a Reaction Mix whenever possible
	Air bubbles formed in well	Pipette gently against the wall of the plate well
	Standard stock is at incorrect concentration	Refer to the standard dilution instructions in the Technical Bulletin
	Calculation errors	Recheck calculations after referring to Technical Bulletin
	Substituting reagents from older kits/lots	Use fresh components from the same kit
Unanticipated results	Samples measured at incorrect wavelength	Check the equipment and filter settings
	Samples contain interfering substances	If possible, dilute sample further
	Sample readings above/below the linear range	Concentrate or dilute samples so readings are in the linear range

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