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Product Information

Sirtuin Activity Assay Kit

Catalog Number **EPI018**
Storage Temperature –20 °C

TECHNICAL BULLETIN

Product Description

Sirtuins are a class of proteins that possess either histone deacetylase or mono-ribosyltransferase activity. Sirtuins are localized in the cytoplasm, nucleus, nucleolus as well as mitochondria. They are associated with aging, cellular protection, sugar metabolism, and cell cycle regulation. 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 that the human sirtuins may function as intracellular regulatory proteins with mono-ADP-ribosyltransferase activity.

In the Sirtuin Activity Assay Kit, the acetylated p53-AFC substrate is deacetylated by sirtuins in the presence of NAD^+ to generate the deacetylated p53-AFC substrate, nicotinamide, and O-Acetyl-ADP Ribose. Cleavage of the deacetylated p53-AFC substrate by the Developer releases the fluorescent group, which is detected fluorometrically at λ_{ex} = 400 nm/λ_{em} = 505 nm. HDACs also deacetylate the acetylated p53-AFC substrate. Trichostatin A is added to the reaction to specifically inhibit HDACs in samples. This kit provides a rapid, simple, sensitive, and reliable test to measure sirtuin activity in a variety of samples. The limit of quantification of the assay is 0.06 μU of recombinant human SIRT6.

The kit is suitable for the detection of sirtuin activity in purified recombinant proteins, cell and tissue lysates, nuclear extracts, mitochondria, and immunoprecipitated samples.

Components

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

Sirtuin Assay Buffer Catalog Number EPI018A	25 mL
Homogenization Buffer Catalog Number EPI018B	100 mL
1 M DTT Catalog Number EPI018C	0.4 mL
Substrate (in DMSO) Catalog Number EPI018D	0.2 mL
NAD Catalog Number EPI018E	1 vial
Positive Control Catalog Number EPI018F	20 μL
Trichostatin A (in DMSO) Catalog Number EPI018G	50 μL
Developer Catalog Number EPI018H	1 mL
AFC Standard (in DMSO) (1 mM) Catalog Number EPI018I	0.1 mL

Reagents and Equipment Required but Not Provided.

- Pipetting devices and accessories (e.g., multichannel pipettor)
- White flatbottom 96 well plates
- Fluorescence multiwell plate reader
- Refrigerated microcentrifuge capable of RCF ≥16,000 × *g*
- Protease Inhibitor Cocktail (Catalog Number P8340)

Precautions and Disclaimer
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.

Storage/Stability
The kit is shipped on wet ice. Store components at –20 °C, protected from light. Briefly centrifuge small vials prior to opening.

Preparation Instructions.
Reagent Preparation

Sirtuin Assay Buffer: Warm to 37 °C and add DTT to a final concentration of 2 mM just before use. Make fresh as needed

Homogenization Buffer: Thaw at room temperature and keep on ice while in use.

Substrate: Aliquot and Store at –20 °C. Avoid repeated freeze/thaw. Use fresh tip each time.

NAD: Reconstitute with 220 µL of ultrapure water. Aliquot and store at –80 °C after reconstituting. Avoid repeated freeze/thaw.

Positive Control: Store at –80 °C. Avoid repeated freeze/thaw cycles.

Trichostatin A: Store at –20 °C. Use fresh tip each time.

Developer: Aliquot and store at –20 °C. Avoid repeated freeze/thaw cycles. Keep on ice while in use.

Procedure
Sample Preparation

Tissue
1. Add DTT to Homogenization Buffer to a final concentration of 2 mM. Make fresh as needed.
2. Rinse tissue and transfer ~100 mg of fresh or frozen tissue (stored at –80 °C) to a pre-chilled tube.
3. Add 600 µL of cold Homogenization Buffer containing protease inhibitor cocktail (not provided) and thoroughly homogenize tissue on ice.
4. Transfer the tissue homogenate to a cold microfuge tube.

Cell Extract
1. Add 150-300 µL of cold Homogenization Buffer containing protease inhibitor cocktail (not provided) to 1–5 × 10^6 fresh or frozen cells and homogenize cells on ice.
2. Transfer the cell homogenate including cell debris to a cold microfuge tube and agitate on a rotary shaker at 4 °C for 15 minutes.
3. Centrifuge the tissue or cell homogenate at 16,000 × *g* for 20 minutes at 4 °C.
4. Transfer the clarified supernatant to a fresh pre-chilled tube and keep on ice.
5. Use lysates immediately to assay for Sirtuin Activity.

Mitochondria can be isolated using Mitochondria Isolation Kit For Tissue (Catalog Number MITOISO1 or equivalent), Mitochondria Isolation Kit For Cultured Cells (Catalog Number MITOISO2 or equivalent), or Mitochondria/Cytosol Fractionation Kit (Catalog Number MIT1000 or equivalent).

Nuclear extracts can be prepared using CelLytic™ NuCLEAR™ Extraction Kit (Catalog Number NXTRACT), Nuclear/Cytosol Fractionation Kit (BioVision Number K266), or equivalent.

Note: Lysates can be aliquoted and snap frozen in liquid nitrogen before storing at –80 °C. Avoid freeze/thaw.

Standard Curve Preparation
Dilute AFC Standard to 10 µM by adding 10 µL of 1 mM AFC Standard to 990 µL of Sirtuin Assay Buffer (with DTT). Prepare AFC Standards in desired wells of a white 96 well plate according to Table 1. Mix well.

Table 1.
Preparation of AFC Standards

Well	10 µM Premix	Sirtuin Assay Buffer (with DTT)	AFC (pmol/well)
1	0 µL	100 µL	0
2	20 µL	88 µL	200
3	40 µL	60 µL	400
4	60 µL	40 µL	600
5	80 µL	20 µL	800
6	100 µL	-	1,000

Sirtuin Activity Assay

1. Add 2–50 µL of cell/tissue homogenate/nuclear extract/mitochondria or purified protein into desired wells in a 96 well plate.
2. For the positive control, add 2 µl of Positive Control in desired well.
3. Make up the volume of samples and Positive Control to 50 µL/well with Sirtuin Assay Buffer (with DTT).

Notes:

- For unknown samples, it is suggested to test several doses to ensure the readings are within the Standard Curve range.
- For samples having HDAC activity, it is recommended using two wells for each sample, one with (1 µL) and the other without Trichostatin A. Measure the Sirtuin Activity and HDAC plus Sirtuin Activity, respectively.
- Use heat inactivated sample as sample background control. For Positive Control Background, use Sirtuin Assay Buffer (with DTT) as background control. Make up the volume to 50 µL/well with Sirtuin Assay Buffer (with DTT).

Reaction Mix

1. Mix enough reagents for the number of assays to be performed. For each well, prepare 40 µL of Reaction Mix according to Table 2.

Table 2.
Preparation of Reaction Mix

Reagent	**Reaction Mix**
Sirtuin Assay Buffer (with DTT)	36 µL
Substrate	2 µL
NAD	2 µL

2. Add 40 µL of Reaction Mix to each test sample, standard curve, positive control, and sample background control wells, mix well.
3. Incubate at 37 °C for 30–60 minutes.
4. After incubation, add 10 µL of Developer to each well except Standards and mix well.
5. Incubate for 10–15 minutes at 37 °C.

Measurement

Measure fluorescence (λ_{ex} = 400 nm/λ_{em} = 505 nm) in end point mode.

Results

1. Subtract the 0 pmol Standard reading from all Standard readings and plot the AFC Standard Curve.
2. Subtract the sample background control reading from Sample reading.
3. Apply the corrected sample reading to the AFC Standard Curve to get B pmol of AFC generated by sirtuin activity in the sample wells.

Sample Sirtuin/HDAC activity (pmol/min/μg or μU/μg) =

$$B / T*S$$

where:
B = AFC amount in the sample well from Standard Curve (pmol)
T = reaction time in minutes (30–60 minutes)
S = sample amount (μg)

Note: Sample sirtuin activity can also be expressed as mU/mg (nmoles/minute deacetylated peptide generated per mg of protein).

Unit Definition: One unit of sirtuin activity is the amount of enzyme that hydrolyzes the substrate to yield 1.0 μmol of AFC/minute at 37 °C.

Figure 1.
Typical AFC Standard Curve

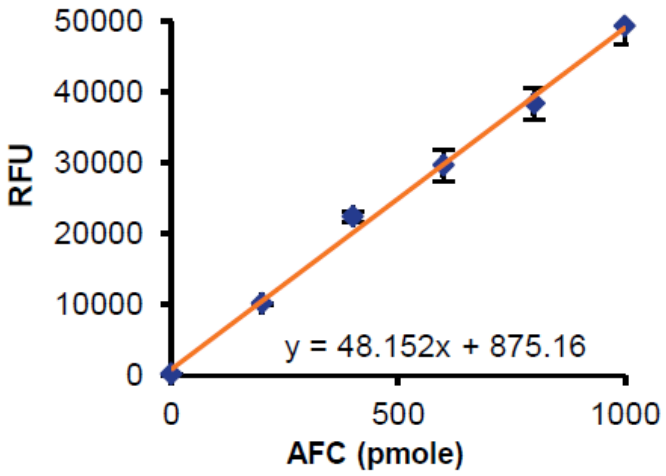
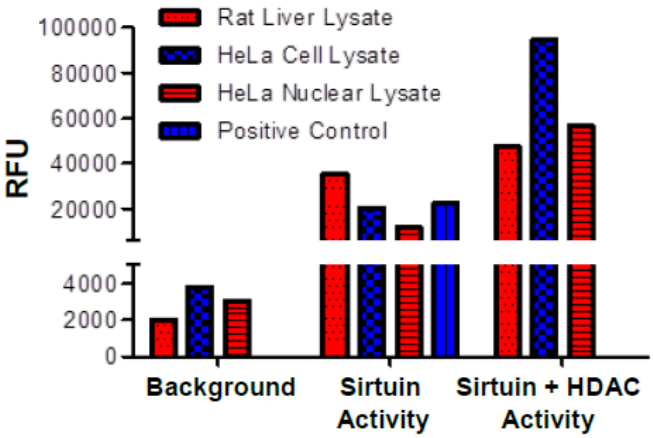
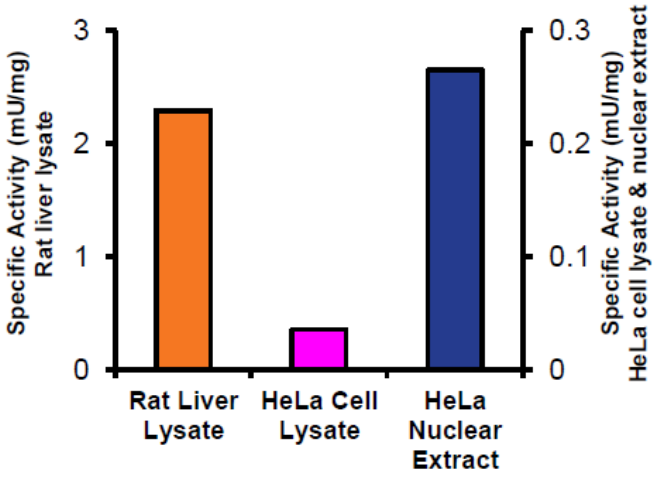


Figure 2.
Sirtuin and Sirtuin plus HDAC activity



Activity in rat liver lysate (5 μg), HeLa cell lysate (160 μg), HeLa nuclear extract (11.5 μg), and Positive Control (2 μL).

Figure 3.
Sirtuin Specific Activity



Sirtuin Specific Activity in samples used in Figure 2. Assays were performed following the kit procedure.

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