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Data Sheet

Fluorogenic SIRT1 (Sir2) Assay Kit

Catalog #: 50081

DESCRIPTION: Sirtuins are NAD+ dependent class III histone deacetylases that regulate important biological processes including metabolism and aging. In human, there are seven isoforms of Sirtuins, SIRT1 to SIRT7. Four of the isoforms, SIRT4 to SIRT7, have no detectable or very weak deacetylase activity. Sirtuin 5 (SIRT5) is shown to be a potent desuccinylase and demalonylase of lysine residues in mitochondrial proteins. The Fluorogenic SIRT1 Assay Kit is a complete assay system designed to measure Sirtuin 1 (SIRT1) activity for screening and profiling applications. It comes in a convenient 96-well format, with all the reagents necessary for 100 fluorescent SIRT1 (Sir2) activity measurements. In addition, the kit includes purified SIRT1 enzyme and a SIRT inhibitor, Nicotinamide, for use as a positive and negative control, respectively. The Fluorogenic SIRT1 Assay Kit is based on a unique fluorogenic substrate and developer combination. This assay method eliminates dealing with the radioactivity, extraction, and chromatography aspects of traditional assays. Using this kit, only two simple steps on a microtiter plate are needed to analyze the SIRT1 activity level. First, the HDAC fluorometric substrate (HDAC substrate 1), containing an acetylated lysine side chain, is incubated with purified SIRT1 enzyme. The desuccinylation sensitizes the substrate so subsequent treatment with the Lysine Developer produces a fluorophore that can then be measured using a fluorescence reader.

COMPONENTS:

Catalog #	Reagent	Amount	Storage	
50012	SIRT1 human recombinant enzyme	50 µg	-80°C	***Avoid freeze/ thaw cycles!***
50032	Fluorogenic HDAC substrate 1 (5 mM)	50 µl	-80°C	
	Nicotinamide Adenine Dinucleotide (NAD^+) (50 mM)	50 µl	-80°C	
	Nicotinamide (10 mM)	500 µl	-80°C	
	2x SIRT Developer (contains 2 mM Nicotinamide)	6 ml	-80°C	
50090	SIRT assay buffer	10 ml	-20°C	
79685	Black, low binding NUNC black microtiter plate	1 plate	Room temp.	

MATERIALS OR INSTRUMENTS REQUIRED BUT NOT SUPPLIED:
BSA (bovine serum albumin) (1 mg/ml)
Fluorescent microplate reader

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APPLICATIONS: Great for studying enzyme kinetics and screening small molecular inhibitors for drug discovery and HTS applications.

STABILITY: One year from date of receipt when stored as directed.

REFERENCES:

1. A. Ito *et al.* (2001) *EMBO J.* **20** 1331.
2. N.A. Barlev *et al.* (2001) *Mol. Cell* **8** 1243.
3. A. Ito *et al.* (2002) *EMBO J.* **21** 6236.

ASSAY PROTOCOL:

Immediately prior to assay:

1) Dilute HDAC substrate 5 mM stock 50-fold with HDAC assay buffer to make a 100 µM solution. (Make only sufficient quantity needed for the assay; store remaining 5 mM stock solution in aliquots at -80°C.)

2) Dilute SIRT1 in HDAC assay buffer to 25 ng/µl (500ng/reaction)*. Aliquot any remaining enzyme and store undiluted at -80°C. Keep diluted enzyme on ice. Discard any remaining diluted enzyme after use. **Note: optimal enzyme concentration may vary with the specific activity of the enzyme.*

Step 1:
Perform all reactions in duplicate.

	Positive Control	Inhibitor Control	Test Inhibitor	"Blank"
Sirt1 substrate (100 µM)	5 µl	5 µl	5 µl	5 µl
BSA (1 mg/ml)	5 µl	5 µl	5 µl	5 µl
NAD^+ (50 mM)	0.5 µl	0.5 µl	0.5 µl	0.5 µl
HDAC assay buffer	14.5 µl	14.5 µl	14.5 µl	34.5 µl
Nicotinamide (10mM)	–	5 µl	–	–
Test Inhibitor	–	–	5 µl	–
Inhibitor buffer (no inhibitor)	5 µl			5 µl
SIRT1 (25 ng/µl)	20 µl	20 µl	20 µl	–
Total	**50 µl**	**50 µl**	**50 µl**	**50 µl**

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Add the reaction mixtures to the black microtiter plate as follows:

1) Prepare the master mixture: N wells × (5 µl diluted **SIRT substrate** (100 µM) + 0.5 µl **NAD^+** + 5 µl BSA (1 mg/ml) + 14.5 µl **SIRT assay buffer**). Add 25 µl of master mixture to all wells.

2) Add 5 µl of inhibitor solution of each well designated “Test Inhibitor”.

3) For the “Positive Control" and “Blank”, add 5 µl of the same solution without inhibitor (inhibitor buffer).

4) Add 5 µl of Nicotinamide (10mM) to the wells designated "Inhibitor Control".

5) Add 20 µl of **SIRT assay buffer** to the wells designated "Blank".

6) Initiate reaction by adding 20 µl of diluted **SIRT1 enzyme** to the wells designated “Positive Control”, "Inhibitor Control", and "Test Inhibitor Control". Incubate at 37ºC for 30 min.

Step2:
Add 50 µl of SIRT assay developer (2x) to each well. Incubate the plate at room temperature for 15 minutes.

Step 3:
Read sample in a microtiter-plate reading fluorimeter capable of excitation at a wavelength in the range of 350-380 nm and detection of emitted light in the range of 440-460 nm. “Blank” value is subtracted from all other values.

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RELATED PRODUCTS:

Fluorogenic SIRT2 Assay Kit	#50082	96 rxns.
Fluorogenic SIRT3 Assay Kit	#50083	96 rxns.
Chemiluminescent SIRT6 Assay Kit	#50086	96 rxns.
SIRT1 (Sir2) Enzyme	#50012	100 µg
SIRT2 Enzyme	#50013	100 µg
SIRT3 Enzyme	#50014	100 µg
SIRT4 Enzyme	#50015	100 µg
SIRT5 Enzyme	#50016	100 µg
SIRT6 Enzyme	#50017	100 µg
SIRT7 Enzyme	#50018	100 µg
SIRT Assay Developer	#50089	6 mL

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