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Data Sheet

Fluorogenic SIRT5 Assay Kit

Catalog #: 50085

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 SIRT5 Assay Kit is a complete assay system designed to measure SIRT5 activity for screening and profiling applications. It comes in a convenient 96-well format, with all the reagents necessary for 100 fluorescent SIRT5 activity measurements. In addition, the kit includes purified SIRT5 enzyme and a SIRT inhibitor, Nicotinamide, for use as a positive and negative control, respectively. The *Fluorogenic SIRT5 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 three simple steps on a microtiter plate are needed to analyze the SIRT5 activity level. First, the Sirtuin 5 fluorometric substrate (SIRT substrate 5), containing a succinylated lysine side chain, is incubated with purified SIRT5 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	
50016	SIRT5 human recombinant enzyme	50 µg	-80°C	***Avoid freeze/ thaw cycles!***
50126	Fluorogenic SIRT5 Substrate (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.	

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

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.

REFERENCE(S):

1. J. Du *et al.* (2011) *Science.* **334:** 806.
2. A. Madsen *et al.* (2012) *J. Med. Chem* **55:** 5582.
3. F. Fisher *et al.* (2012) *Plos One.* **7:** 1.

ASSAY PROTOCOL:

Immediately prior to assay:

1) Dilute 5 mM SIRT5 substrate stock 50-fold with SIRT 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 SIRT5 in SIRT assay buffer to 15 ng/µl (300 ng/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"
Sirt5 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
SIRT 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
SIRT5 (15 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 **SIRT5 enzyme** to the wells designated “Positive Control”, "Inhibitor Control", and "Test Inhibitor Control". Incubate at 37ºC for 30 min.

Step 2:
Add 50 µl of undiluted 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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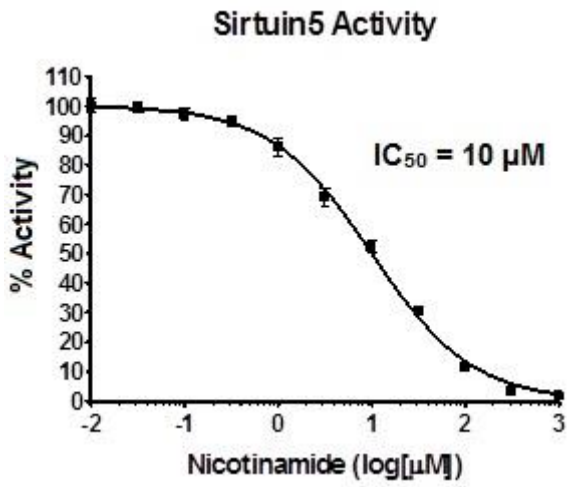
Example of Assay Results:



Sirtuin 5 enzyme activity, measured using the *Fluorogenic SIRT5 Assay Kit*, BPS Bioscience Catalog #50085. Fluorescence was measured using a Tecan fluorescent microplate reader. *Data shown is lot-specific. For lot-specific information, please contact BPS Bioscience, Inc. at info@bpsbioscience.com.*

RELATED PRODUCTS:

Product Name	Catalog	Size
Fluorogenic SIRT1 Assay Kit	#50081	96 rxns.
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
SIRT Assay Buffer	#50090	20 mL

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