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Data Sheet

Fluorogenic SIRT6 Assay Kit

Catalog #: 50022

BACKGROUND: 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 6 (SIRT6) is shown to be a potent defatty-acylase of lysine residues.

DESCRIPTION: *The Fluorogenic SIRT6 Assay Kit* is a complete assay system designed to measure SIRT6 activity for screening and profiling applications. It comes in a convenient 96-well format, with all the reagents necessary for 100 fluorescent SIRT6 activity measurements. In addition, the kit includes purified SIRT6 enzyme and a SIRT inhibitor, Nicotinamide, for use as a positive and negative control, respectively. The *Fluorogenic SIRT6 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 SIRT6 activity level. First, the Sirtuin 6 fluorometric substrate, containing a myristoylated lysine side chain, is incubated with purified SIRT6 enzyme. The demyristoylation sensitizes the substrate so subsequent treatment with the SIRT Developer produces a fluorophore that can then be measured using a fluorescence reader.

COMPONENTS:

Catalog #	Reagent	Amount	Storage	
50017	SIRT6 human recombinant enzyme	100 µg	-80°C	***Avoid freeze/ thaw cycles!***
50866	Fluorogenic SIRT6 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.

REFERENCES:
Hu, J. *et al., Org Miomol Chem*, 2013. **11(32):**5213-5216.

ASSAY PROTOCOL:

Immediately prior to assay:

1) Dilute **Fluorogenic SIRT6 substrate (5 mM)** 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 **SIRT6 human recombinant enzyme** in **SIRT assay buffer** to 50 ng/µl (1000 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"**
SIRT6 substrate 2 (100 µM)	5 µl	5 µl	5 µl	5 µl
NAD^+ (50 mM)	0.5 µl	0.5 µl	0.5 µl	0.5 µl
BSA (1 mg/ml)	5 µl	5 µl	5 µl	5 µl
SIRT assay buffer	14.5 µl	14.5 µl	14.5 µl	34.5 µl
Nicotinamide (10 mM)	–	5 µl	–	–
Test Inhibitor	–	–	5 µl	–
10% DMSO in water (inhibitor buffer)	5 µl			5 µl
SIRT6 (50 ng/µl)	20 µl	20 µl	20 µl	–
Total	**50 µl**	**50 µl**	**50 µl**	**50 µl**

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1. In duplicate, add the reaction mixtures (below) to the microtiter black plate as follows:

1) Prepare the master mixture: N wells × {(5 µl **SIRT6 substrate 2***) + 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." For the "Positive Control " and "Blank," add 5 µl of 10% DMSO in water (inhibitor buffer). Add 5 µl of Nicotinamide (10 mM) *to the well designated "Inhibitor Control."*

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

4) *Initiate reaction by adding 20 µl of diluted* **SIRT6 enzyme** *to the wells designated "Positive Control," "Inhibitor Control," and "Test Inhibitor Control," Incubate at 37ºC for 60 min.*

Step 2:
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 of 360 nm and detection of emitted light at 460 nm. "Blank" value is subtracted from all other values.

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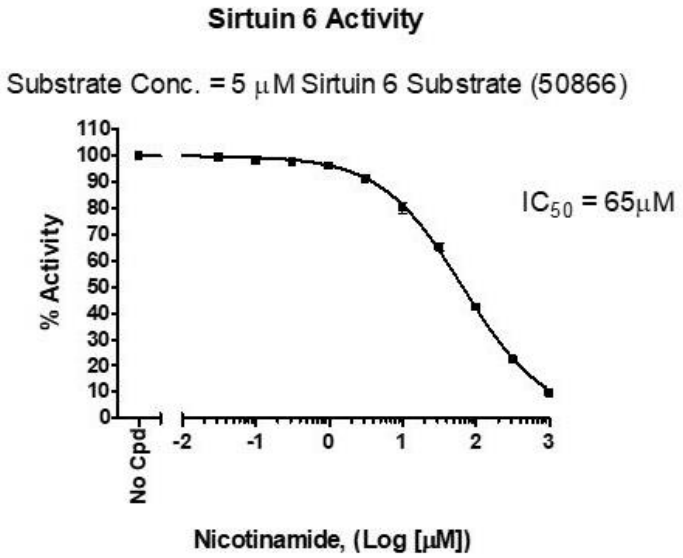
Example of Assay Results:



Inhibition of SIRT6 enzyme by Nicotinamide, measured using the Fluorogenic SIRT6 Assay Kit (Cat. #50022). *Data shown is lot-specific. For lot-specific information, please contact BPS Bioscience, Inc. at info@bpsbioscience.com*

RELATED PRODUCTS:

Product Name	**Catalog #**	**Size**
Sirtuin 6, GST-tag	50017	100 µg
Sirtuin Developer (2X)	50089	6 ml
Sirtuin Assay Buffer	50090	20 ml
Fluorogenic SIRT substrate 1	50080	500 nmol

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