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Data Sheet

Fluorogenic SIRT3 Assay Kit

Catalog #: 50088

DESCRIPTION: The *Fluorogenic SIRT3 Assay Kit* is a complete assay system designed to measure Sirtuin 3 (SIRT3) activity for screening and profiling applications. It comes in a convenient 96-well format, with all the reagents necessary for **32 fluorescent SIRT3 activity measurements***. The kit includes purified SIRT3 enzyme and a SIRT inhibitor, Nicotinamide, for use as a positive and negative control, respectively. The *Fluorogenic SIRT3 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 SIRT3 activity level. First, the HDAC fluorometric substrate (HDAC substrate 1) is incubated with purified SIRT3 enzyme. The deacetylation sensitizes the substrate so subsequent treatment with the Lysine Developer produces a fluorophore that can then be measured using a fluorescence reader.

**Note: The kit includes sufficient HDAC substrate 1, buffers, NAD+, nicotinamide, and detection reagents for a full 96-well plate. Researchers can test their own SIRT3-containing samples in the remaining wells, or additional SIRT3 enzyme may be ordered separately (Cat. #50014).*

COMPONENTS:

Catalog #	Component	Amount	Storage	
50014	SIRT3 human recombinant enzyme	70 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.	

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

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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 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 SIRT3 in SIRT assay buffer to 100 ng/µl (2000 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:

In duplicate, add the reaction mixtures (below) to the microtiter black plate. Incubate at 37ºC for 60 min.

	Enzyme Positive Control	**Inhibitor Negative Control**	**Test Inhibitor**	**"Blank" Negative Control**
SIRT3 (100 ng/µl)	20 µl	20 µl	20 µl	–
HDAC 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
Nicotinamide (10mM)	–	5 µl	–	–
Test Inhibitor	–	–	X µl	–
SIRT assay buffer	19.5 µl	14.5 µl	19.5 - X µl	40 µl
Total	**50 µl**	**50 µl**	**50 µl**	**50 µl**

Step2:

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

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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.

RELATED PRODUCTS:

Fluorogenic SIRT1 Assay Kit	#50081	96 rxns.
Fluorogenic SIRT2 Assay Kit	#50082	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