

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), containing an acetylated lysine side chain, is incubated with a sample containing SIRT3 activity (e.g. HeLa nuclear extract or 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:

	Cat. #	Reagent	Amount	Storage
Avoid freeze/ thaw cycles!	50014	SIRT3 human recombinant enzyme	70 µg	-80°C
	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
	50031	HDAC assay buffer	10 ml	-20°C
		black, low binding NUNC black microtiter plate	1 plate	Room temp.

OUR PRODUCTS ARE FOR RESEARCH USE ONLY. NOT FOR DIAGNOSTIC OR THERAPEUTIC USE.



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

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 μ M solution. (Make only sufficient quantity needed for the assay; store remaining 5 mM stock solution in aliquots at -80 °C.)
- 2) Dilute SIRT3 in HDAC assay buffer to 100 ng/ μ l (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 37 °C for 30 min.

	Enzyme Positive Control	Inhibitor Negative Control	Test Inhibitor	“Blank” Negative Control
SIRT3 (100 ng/ μ l)	20 μ l	20 μ l	20 μ l	–
HDACsubstrate (100 μ M)	5 μ l	5 μ l	5 μ l	5 μ l
BSA (1 mg/ml)	5 μ l	5 μ l	5 μ l	5 μ l
NAD ⁺ (50 mM)	0.5 μ l	0.5 μ l	0.5 μ l	0.5 μ l
Nicotinamide (10mM)	–	5 μ l	–	–
Test Inhibitor	–	–	X μ l	–
HDAC assay buffer	19.5 μ l	14.5 μ l	19.5 - X μ l	40 μ l
Total	50 μl	50 μl	50 μl	50 μl

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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.

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

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