

## Data Sheet

### ***Fluorogenic SIRT3 Assay Kit***

Catalog #: 50088

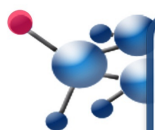
**DESCRIPTION:** The *Fluorogenic SIRT3 Assay Kit* is a complete assay system designed to measure Sirtuin 3 (SIRT3) activity for screening and profiling applications. It comes in a convenient 96-well format, with all the reagents necessary for **32 fluorescent SIRT3 activity measurements\***. The kit 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<sup>+</sup>, 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
<b>Avoid freeze/thaw cycles!</b>	50014	SIRT3 human recombinant enzyme	70 µg	-80 °C
	50032	Fluorogenic HDAC substrate 1 (5 mM)	50 µl	-80 °C
		Nicotinamide Adenine Dinucleotide (NAD <sup>+</sup> ) (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.

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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  $\mu$ 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/ $\mu$ 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/ $\mu$ l)	20 $\mu$ l	20 $\mu$ l	20 $\mu$ l	–
HDACsubstrate (100 $\mu$ M)	5 $\mu$ l	5 $\mu$ l	5 $\mu$ l	5 $\mu$ l
BSA (1 mg/ml)	5 $\mu$ l	5 $\mu$ l	5 $\mu$ l	5 $\mu$ l
NAD <sup>+</sup> (50 mM)	0.5 $\mu$ l	0.5 $\mu$ l	0.5 $\mu$ l	0.5 $\mu$ l
Nicotinamide (10mM)	–	5 $\mu$ l	–	–
Test Inhibitor	–	–	X $\mu$ l	–
HDAC assay buffer	19.5 $\mu$ l	14.5 $\mu$ l	19.5 - X $\mu$ l	40 $\mu$ l
<b>Total</b>	<b>50 <math>\mu</math>l</b>	<b>50 <math>\mu</math>l</b>	<b>50 <math>\mu</math>l</b>	<b>50 <math>\mu</math>l</b>

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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.

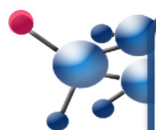
**Step 3:**

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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