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## Data Sheet

### **Fluorogenic SIRT2 Assay Kit**

Catalog #: 50087

**DESCRIPTION:** The *Fluorogenic SIRT2 Assay Kit* is a complete assay system designed to measure Sirtuin 2 (SIRT2) activity for screening and profiling applications. It comes in a convenient 96-well format, with all the reagents necessary for **32 fluorescent SIRT2 activity measurements\***. The kit includes purified SIRT2 enzyme and a SIRT inhibitor, Nicotinamide, for use as a positive and negative control, respectively. The *Fluorogenic SIRT2 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 SIRT2 activity level. First, the HDAC fluorometric substrate (HDAC substrate 1), containing an acetylated lysine side chain, is incubated with purified SIRT2 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 SIRT2-containing samples in the remaining wells, or additional SIRT2 enzyme may be ordered separately (Cat. #50013).*

#### **COMPONENTS:**

Catalog #	Component	Amount	Storage	
50013	SIRT2 human recombinant enzyme	135 µg	-80°C	<b>Avoid freeze/ thaw cycles!</b>
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	
50090	SIRT assay buffer	10 ml	-20°C	
	black, low binding NUNC black microtiter plate	1 plate	Room temp.	

#### **MATERIALS OR INSTRUMENTS REQUIRED BUT NOT SUPPLIED:**

BSA (bovine serum albumin) (1 mg/ml)  
Fluorescent microplate reader  
Adjustable micropipettor and sterile tips  
Rotating or rocker platform

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**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:**

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  $\mu$ M solution. (Make only sufficient quantity needed for the assay; store remaining 5 mM stock solution in aliquots at  $-80^{\circ}\text{C}$ .)
- 2) Dilute SIRT2 in SIRT assay buffer to 200 ng/ $\mu$ l (4000 ng/reaction)\*. Aliquot any remaining enzyme and store undiluted at  $-80^{\circ}\text{C}$ . 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  $37^{\circ}\text{C}$  for 30 min.

Reagent	Enzyme Positive Control	Inhibitor Negative Control	Test Inhibitor	"Blank" Negative Control
SIRT2 (200 ng/ $\mu$ l)	20 $\mu$ l	20 $\mu$ l	20 $\mu$ l	-
HDAC substrate (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 (10 mM)	-	5 $\mu$ l	-	-
Test Inhibitor	-	-	X $\mu$ l	-
SIRT 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  $\mu$ l 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:**

<u>Product Name</u>	<u>Catalog #</u>	<u>Size</u>
Fluorogenic SIRT1 Assay Kit	50081	96 rxns.
Fluorogenic SIRT3 Assay Kit	50088	32 rxns.
Chemiluminescent SIRT6 Assay Kit	50086	96 rxns.
SIRT1 (Sir2) Enzyme	50012	100 $\mu$ g
SIRT2 Enzyme	50013	100 $\mu$ g
SIRT3 Enzyme	50014	100 $\mu$ g
SIRT4 Enzyme	50015	100 $\mu$ g
SIRT5 Enzyme	50016	100 $\mu$ g
SIRT6 Enzyme	50017	100 $\mu$ g
SIRT7 Enzyme	50018	100 $\mu$ g
SIRT Assay Developer	50089	6 mL

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