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Data Sheet

Sirtuin 6 (SIRT6) Chemiluminescent Assay Kit

Catalog # 50086

DESCRIPTION: The *SIRT6 Chemiluminescent Activity Assay kit* is designed for screening and profiling Sirtuin6 inhibitors by measuring autoribosylation of GST-SIRT6 fusion protein. The nuclear protein SIRT6 is a weak deacetylase but is endowed with a robust ADP-ribosyltransferase activity. The *SIRT6 Chemiluminescent Assay Kit* comes in a convenient 96-well format, with Sirtuin assay buffer, activated DNA, and purified SIRT6 enzyme for up to 100 enzyme reactions. The key to the *SIRT6 Chemiluminescent Assay Kit* is the biotinylated substrate. With this kit, only three simple steps are required for SIRT6 reactions. First, GST-SRT6 is coated on a 96-well glutathione-precoated plate. Next, the SIRT6 biotinylated substrate is added to the wells. Finally, the plate is treated with streptavidin-HRP followed by addition of the HRP substrate to produce chemiluminescence that can then be measured using a chemiluminescence reader.

COMPONENTS:

Catalog #	Component	Amount	Storage	
50017	Sirtuin 6 (SIRT6)	100 µg	-80 °C	Avoid freeze/ thaw cycles!
	20x Assay mixture containing biotinylated substrate	250 µl	-80 °C	
50090	10x Sirtuin assay buffer	1.5 ml	-20 °C	
	Blocking buffer	25 ml	+4 °C	
	Streptavidin-HRP	100 µl	+4 °C	
	HRP chemiluminescent substrate (2 components)	6 ml each	+4 °C	
	96-well glutathione-precoated plate	1	+4 °C	

MATERIALS REQUIRED BUT NOT SUPPLIED:

1x PBS buffer
PBST buffer (1x PBS, containing 0.05% Tween20)
Luminometer or fluorescent microplate reader capable of reading chemiluminescence
Adjustable micropipettor and sterile tips
Rotating or rocker platform

STABILITY: Up to 1 year when stored as recommended.

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

REFERENCE: Brown JA, Marala RB. *J. Pharmacol. Toxicol. Methods* 2002 **47**:137-41.

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Assay Protocol:

Step 1: Coat 50 µl of SIRT6 solution onto a black glutathione-coated plate

Prepare 1x Sirtuin assay buffer by mixing 1 part 10x Sirtuin assay buffer and 9 parts of distilled water (v/v).

1. Dilute enzyme to 20 ng/µl with 1x Sirtuin buffer.
2. Add 50 µl of 1x Sirtuin assay buffer to the control wells and 50 µl of diluted SIRT6 enzyme to the sample wells and incubate at 4°C overnight¹.
3. Wash the plate three times with PBST buffer (1x PBS containing 0.05% Tween-20).
4. Block the wells by adding 150 µl of Blocking buffer to every well. Incubate at room temperature for 30 min.
5. Wash plate three times with PBST buffer as above.

Step 2: Ribosylation reaction

All samples and controls should be tested in duplicate.

1. Add 5 µl of Inhibitor solution of each well labeled as “Test Inhibitor” on the SIRT6-coated plate (above). For the wells labeled “Positive Control” and “Substrate Control”, add 5 µl of the same solution without inhibitor (Inhibitor buffer).
2. Prepare the master mixture: N wells x (5 µl 10x Sirtuin buffer + 2.5 20x Assay mixture + 37.5 µl water). Add 42.5 µl to wells designated “Positive Control” and “Test Sample”. To wells labeled “Substrate Control”, add 5 µl 10x Sirtuin buffer + 40 µl water

	Positive Control	Substrate Control	Test Sample
Test Inhibitor/Activator	–	–	5 µl
Inhibitor Buffer (no inhibitor)	5 µl	5 µl	–
10x Sirtuin buffer	5 µl	5 µl	5 µl
H ₂ O	37.5 µl	40 µl	37.5 µl
20x Assay mixture	2.5 µl	–	2.5 µl
Total	50 µl	50 µl	50 µl

¹ Alternately, incubate 1 hour at 37°C.

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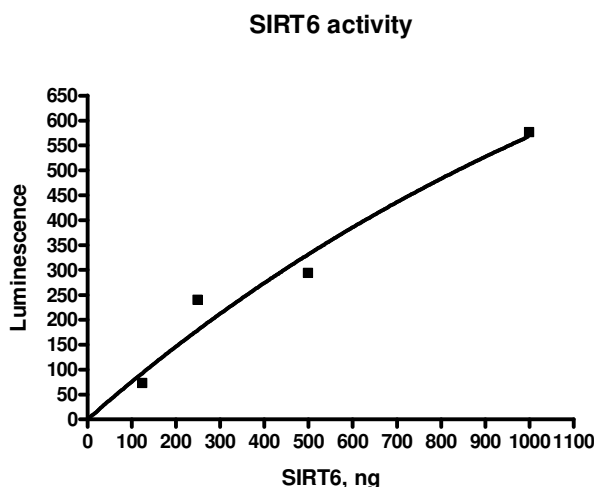
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3. Incubate at 30°C for 1 hour.
4. Wash plate three times with PBST buffer as above.

Step 3: Detection

1. Dilute Streptavidin-HRP 1:50 in Blocking buffer.
2. Add 50 µl of diluted Streptavidin-HRP to each well. Incubate for 30 min. at room temperature.
3. Wash three times with PBST buffer as above.
4. Just before use, mix on ice 50 µl HRP chemiluminescent substrate A and 50 µl HRP chemiluminescent substrate B and add 100 µl per well. Incubate at room temperature for 10 min.
5. Read sample in a luminometer or microtiter-plate capable of reading chemiluminescence.

Example of Assay Results:



Sirtuin6 enzyme activity, measured using the Sirtuin6 Chemiluminescent Assay Kit, BPS Bioscience Cat. #50086. Luminescence was measured using a Bio-Tek fluorescent microplate reader. *Data shown is lot-specific. For lot-specific information, please contact BPS Bioscience, Inc. at info@bpsbioscience.com.*

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RELATED PRODUCTS:

<u>Product</u>	<u>Cat. #</u>	<u>Size</u>
Sirt6 Enzyme	#50017	100 µg
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
Sirt7 Enzyme	#50018	100 µg
HDAC Assay Kit	#50033	96 rxns.
Fluorogenic SIRT1 Assay Kit	#50081	96 rxns.
Fluorogenic SIRT2 Assay Kit	#50088	32 rxns.
Fluorogenic SIRT3 Assay Kit	#50087	32 rxns.
Fluorogenic SIRT5 Assay Kit	#50085	96 rxns.
Sirtuin Assay Buffer	#50090	20 mL
SIRT Assay Developer	#50089	6 mL
Fluorogenic SIRT Substrate	#50080	500 nmole

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