（FOR RESEARCH USE ONLY. DO NOT USE IT IN CLINICAL DIAGNOSIS !）

Catalog No：E-BC-F056

Specification：48T(30 samples)/96T(78 samples)

Measuring instrument：Fluorescence Microplate Reader

Detection range：0.03–9.66 U/L

Elabscience® Sirtuin 1 (SIRT-1) Activity Assay Kit

This manual must be read attentively and completely before using this product.

If you have any problem, please contact our Technical Service Center for help：

Toll-free: 1-888-852-8623

Tel: 1-832-243-6086

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Email: techsupport@elabscience.com

Website: www.elabscience.com

Please kindly provide us the lot number (on the outside of the box) of the kit for more efficient service.

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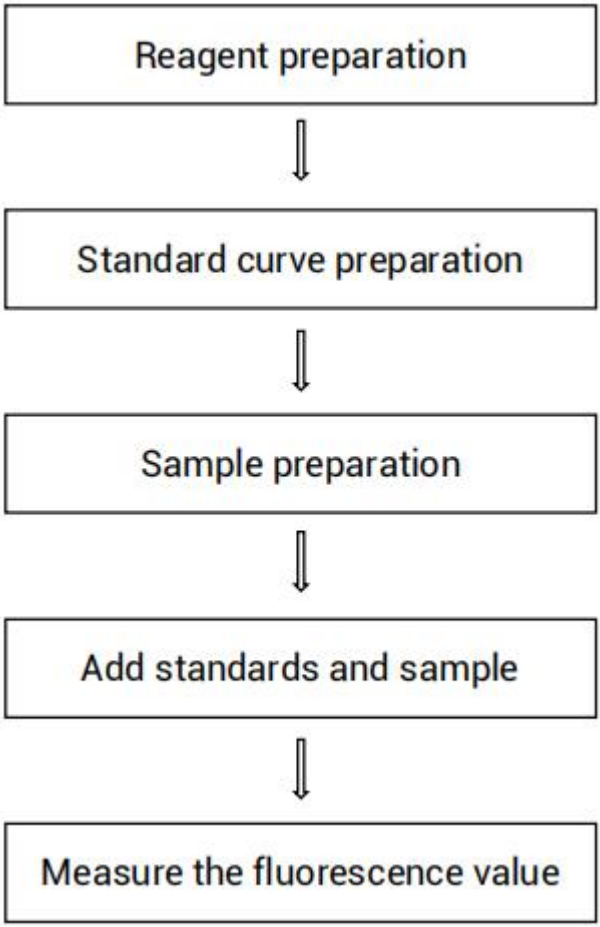
Assay summary



Intended use

This kit can be used to measure SIRT-1 activity in animal tissue and cell samples.

Detection principle

The Sirtuins (SIRT) family of proteins includes SIRT-1 and SIRT-7, which are histone deacetylases with nicotinamide adenine dinucleotide (NAD^+) dependence and are commonly expressed in organs and tissues. Based on the amino acid sequence, the Sirtuins family proteins can be divided into four types: SIRT-1 and SIRT-3 are type 1, SIRT-4 is type 2, SIRT-5 is type 3, and SIRT-6 and SIRT-7 are type 4. SIRT-1 is widely distributed in organs and tissues, mainly present in the cytoplasm, and needs to enter the nucleus to function. Some drugs can regulate the expression of certain genes by inhibiting SIRT-1 entry into the nucleus.

This kit uses the fluorescence resonance energy transfer (FRET) method for detection. After deacetylation by SIRT-1, the fluorescent substrate generates an activated fluorescent substrate, which can be decomposed by proteases to produce fluorescent substances. The maximum excitation wavelength of fluorescent substances is 340 nm, and the maximum emission wavelength is 440 nm.

Kit components & storage

Item	Component	Size 1 (48 T)	Size 2 (96 T)	Storage
Reagent 1	Buffer Solution	7.5 mL × 1 vial	15 mL × 1 vial	-20℃, 12 months
Reagent 2	Substrate	0.075 mL × 1 vial	0.15 mL × 1 vial	-20℃, 12 months, shading light
Reagent 3	Accelerant	Powder × 1 vial	Powder × 2 vials	-20℃, 12 months, shading light
Reagent 4	Activator	1.4 mL × 1 vial	1.4 mL × 2 vials	-20℃, 12 months, shading light
Reagent 5	1mmol/L Standard Solution	0.25 mL × 1 vial	0.5 mL × 1 vial	-20℃, 12 months, shading light
	Black Microplate	96 wells		No requirement
	Plate Sealer	2 pieces		
	Sample Layout Sheet	1 piece		

Note: The reagents must be stored strictly according to the preservation conditions in the above table. The reagents in different kits cannot be mixed with each other. For a small volume of reagents, please centrifuge before use, so as not to obtain sufficient amount of reagents.

Materials prepared by users

Instruments:

Fluorescence microplate reader (Ex/Em=340 nm/440 nm), Incubator

Reagents:

Normal saline (0.9% NaCl), Double distilled water

Reagent preparation

① Equilibrate all reagents to room temperature before use.

② Preparation of accelerant working solution：

Dissolve one vial of accelerant with 1 mL of double distilled water, mix well to dissolve. Store at -20°C for 2 weeks protected from light.

③ Preparation of reaction working solution：

For each well, prepare 102 μL of reaction working solution (mix well 100 μL of buffer solution, 1 μL of substrate and 2 μL of accelerant working solution). The prepared solution should be prepared on spot. Keep reaction working solution protected from light on ice during use. The prepared solution should be used up within 2 h.

④ Preparation of 100 μmol/L standard solution：

Dilute 0.05 mL of 1mmol/L standard solution with 0.45 mL of double distilled water, mix well to dissolve. The prepared solution should be prepared on spot. Keep 100 μmol/L standard solution protected from light on ice during use. The prepared solution should be used up within the same day.

⑤ The preparation of standard curve：

Always prepare a fresh set of standards. Discard working standard dilutions after use.

Dilute 100 μmol/L standard solution with double distilled water to a serial

concentration. The recommended dilution gradient is as follows: 0, 20, 30, 40, 60, 80, 90, 100 μmol/L. Reference is as follows:

Item	①	②	③	④	⑤	⑥	⑦	⑧
Concentration (μmol/L)	**0**	**20**	**30**	**40**	**60**	**80**	**90**	**100**
100 μmol/L standard (μL)	0	20	30	40	60	80	90	100
Double distilled water (μL)	100	80	70	60	40	20	10	0

Sample preparation

① Sample preparation

Tissue sample:

① Harvest the amount of tissue needed for each assay (initial recommendation 20 mg).

② Wash tissue in cold PBS (0.01 M, pH 7.4).

③ Homogenize 20 mg tissue in 180 μL normal saline (0.9% NaCl) with a dounce homogenizer at 4ºC.

④ Centrifuge at 10000×g for 10 min at 4ºC to remove insoluble material. Collect supernatant and keep it on ice for detection.

⑤ Meanwhile, determine the protein concentration of supernatant (E-BC-K318-M).

Cell (adherent or suspension) samples:

① Harvest the number of cells needed for each assay (initial recommendation 1×10^6 cells).

② Wash cells with PBS (0.01 M, pH 7.4).

③ Homogenize 1×10^6 cells in 200 μL normal saline (0.9% NaCl) with a ultrasonic cell disruptor at 4ºC.

④ Centrifuge at 10000×g for 10 min at 4ºC to remove insoluble material. Collect supernatant and keep it on ice for detection.

⑤ Meanwhile, determine the protein concentration of supernatant (E-BC-K318-M).

② **Dilution of sample**

The recommended dilution factor for different samples is as follows (for reference only):

Sample type	Dilution factor
10% Rat liver tissue homogenate	1
10% Rat lung tissue homogenate	1
10% Mouse brain tissue homogenate	1
1×10^6 CHO cell	1

Note: The diluent is normal saline (0.9% NaCl). For the dilution of other sample types, please do pretest to confirm the dilution factor.

The key points of the assay

① The sample should not contain protease inhibitors, otherwise it will affect the assay.

② Detect the samples within 4 h after processing.

Operating steps

① Standard well: add 5 μL of standard with different concentrations into the well.
Sample well: add 5 μL of sample into the well.
Control well: add 5 μL of normal saline (0.9% NaCl) into the well.

② Add 100 μL of buffer solution into standard well. Add 100 μL of reaction working solution into sample well and control well.

③ Mix fully with microplate reader for 3 s and incubate at 37℃ for 20 min.

④ Add 20 μL of activator into each wells.

⑤ Mix fully with microplate reader for 3 s and incubate at 37℃ for 15 min.

⑥ Measure the fluorescence intensity at the excitation wavelength of 340 nm and the emission wavelength of 440 nm.

Calculation

The standard curve:

1. Average the duplicate reading for each standard.

2. Subtract the mean F value of the blank (Standard #①) from all standard readings. This is the absoluted F value.

3. Plot the standard curve by using absoluted F value of standard and correspondent concentration as y-axis and x-axis respectively. Create the standard curve (**y = ax + b**) with graph software (or EXCEL).

The sample:

Tissue and cell sample:

Definition: The amount of SIRT-1 in 1 g tissue or cell protein per minute that catalyze the substrate to produce 1 μmol AMC at 37℃ is defined as 1 unit.

$$\text{SIRT-1 activity (U/gprot)} = (\triangle F - b) \div a \div T \div C_{pr} \times f$$

[Note]

△F: $F_{Sample} - F_{Control.}$

T: Enzymatic acetylation reaction time, 20 min.

C_{pr}: The concentration of protein in sample, gprot/L

f: Dilution factor of sample before tested.

Appendix I Performance Characteristics

1. Parameter:

Intra-assay Precision

Three rat liver samples were assayed in replicates of 20 to determine precision within an assay (CV = Coefficient of Variation).

Parameters	Sample 1	Sample 2	Sample 3
Mean (U/L)	0.04	0.08	0.15
%CV	5.00	3.00	4.00

Inter-assay Precision

Three human serum samples were assayed 20 times in duplicate by three operators to determine precision between assays.

Parameters	Sample 1	Sample 2	Sample 3
Mean (U/L)	0.11	0.17	0.34
%CV	9.60	8.90	8.40

Recovery

Take three samples of high concentration, middle concentration and low concentration to test the samples of each concentration for 6 times parallelly to get the average recovery rate of 99%.

	Standard 1	Standard 2	Standard 3
Expected Conc. (μmol/L)	0.04	0.08	0.16
Observed Conc. (μmol/L)	0.04	0.08	0.15
Recovery rate (%)	100	105	94

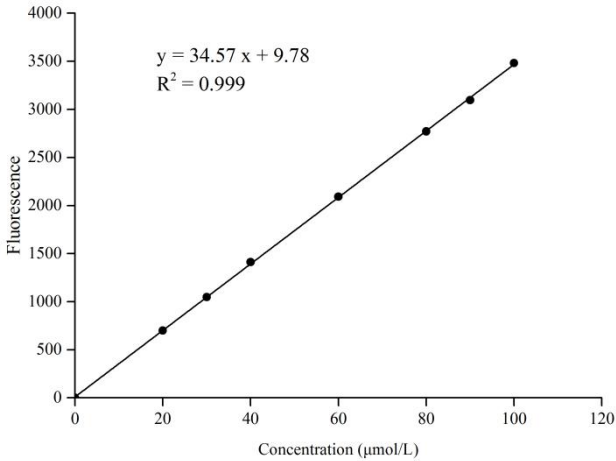
Sensitivity

The analytical sensitivity of the assay is 0.03 U/L. This was determined by adding two standard deviations to the mean O.D. obtained when the zero standard was assayed 20 times, and calculating the corresponding concentration.

2. Standard curve:

As the F value of the standard curve may vary according to the conditions of the actual assay performance (e.g. operator, pipetting technique or temperature effects), so the standard curve and data are provided as below for reference only：

Concentration (μmol/L)	0	20	30	40	60	80	90	100
F	26	717	1086	1413	2073	2736	3045	3474
	16	723	1050	1455	2154	2847	3189	3531
Average F	21	720	1068	1434	2114	2792	3117	3503
Absoluted F	0	699	1047	1413	2092	2770	3096	3481



Appendix Π Example Analysis

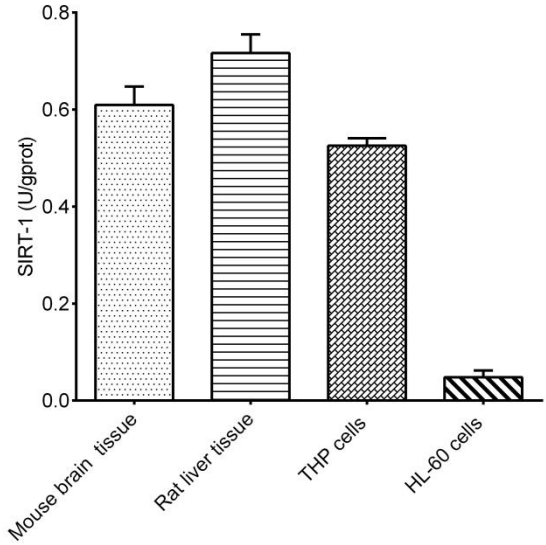
Example analysis：

For 10% mouse brain tissue homogenate, take 5 μL of sample and carry the assay according to the operation table. The results are as follows:

Standard curve: y = 34.57 x + 9.78, the average fluorescence value of the sample is 2017, the average fluorescence value of the control is 1270, △F = 2017 - 1270 = 747, the concentration of protein in the sample is 1.78 gprot/L, and the calculation result is:

$$\text{SIRT-1 activity (U/gprot)} = (747 - 9.78) \div 34.57 \div 20 \div 1.78 = 0.60 \text{ U/gprot}$$

Detect 10% mouse brain tissue homogenate (the concentration of protein is 1.78 gprot/L), 10% rat liver tissue homogenate (the concentration of protein is 8.15 gprot/L), 1×10^6 THP cell (the concentration of protein is 2.21 gprot/L), 1×10^6 HL-60 cell (the concentration of protein is 2.94 gprot/L) according to the protocol, the result is as follows:



Statement

1. This assay kit is for Research Use Only. We will not response for any arising problems or legal responsibilities causing by using the kit for clinical diagnosis or other purpose.
2. Please read the instructions carefully and adjust the instruments before the experiments. Please follow the instructions strictly during the experiments.
3. Protection methods must be taken by wearing lab coat and latex gloves.
4. If the concentration of substance is not within the detection range exactly, an extra dilution or concentration should be taken for the sample.
5. It is recommended to take a pre-test if your sample is not listed in the instruction book.
6. The experimental results are closely related to the situation of reagents, operations, environment and so on. Elabscience will guarantee the quality of the kits only, and NOT be responsible for the sample consumption caused by using the assay kits. It is better to calculate the possible usage of sample and reserve sufficient samples before use.