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

Catalog No：E-BC-K906-M

Specification：48T(32 samples)/96T(80 samples)

Measuring instrument：Microplate reader（440 - 460 nm）

Detection range：0.09-20.72 U/L

Elabscience® Succinyl-CoA Synthetase (SCS) 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
Tell: 1-832-243-6086
Fax: 1-832-243-6017
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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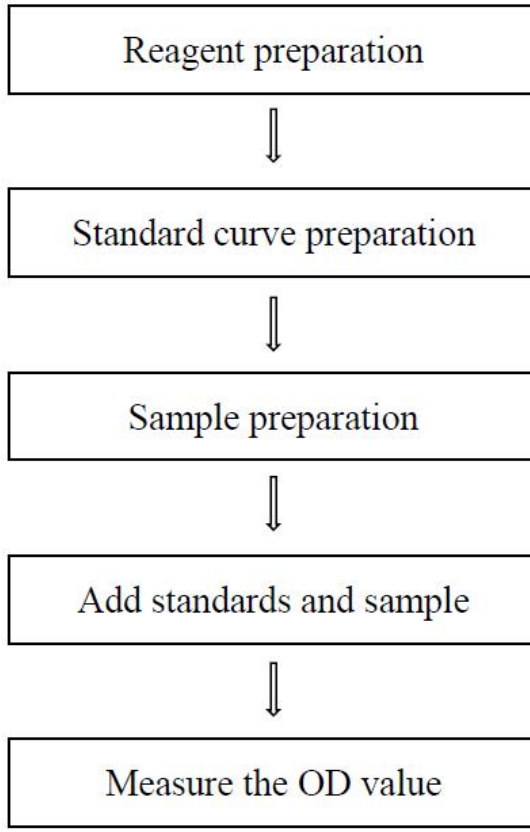
Assay summary



Intended use

This kit can be used to measure succinyl-CoA synthetase (SCS) activity in tissue and cell samples.

Detection principle

Succinyl-CoA synthetase (SCS) is a key enzyme in the citric acid cycle and an important metabolic intermediate in the biosynthesis of porphyrins, hemes, and ketones. SCS converts succinyl-coenzyme A (CoA-SH) to succinic acid. The reaction between CoA-SH and pyruvate produces chromogenic substances with a characteristic absorption peak at 450 nm. The activity of SCS can be calculated by measuring the change of absorbance value at 450 nm.

Kit components & storage

Item	Component	Size 1(48 T)	Size 2(96 T)	Storage
Reagent 1	Buffer Solution	12 mL ×1 vial	24 mL ×1 vial	-20℃, 12 months, shading light
Reagent 2	Co-factor	2.5 mL ×1 vial	5 mL ×1 vial	-20℃, 12 months, shading light
Reagent 3	Substrate	0.8 mL ×1 vial	1.6 mL ×1 vial	-20℃, 12 months, shading light
Reagent 4	Enzyme Solution	0.5 mL ×1 vial	1 mL ×1 vial	-20℃, 12 months, shading light
Reagent 5	Accelerant	0.5 mL ×1 vial	1 mL ×1 vial	-20℃, 12 months, shading light
Reagent 6	Chromogenic Agent	1.5 mL ×1 vial	1.5 mL ×2 vials	-20℃, 12 months, shading light
Reagent 7	Standard	Powder ×1 vial	Powder ×2 vials	-20℃, 12 months, shading light
	Microplate	48 wells	96 wells	No requirement
	Plate Sealer	2 pieces		

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:

Microplate reader (440-460 nm, optimum wavelength: 450 nm), Vortex mixer

Reagents:

Double distilled water, PBS (0.01 M, pH 7.4)

Reagent preparation

① Equilibrate all reagents to room temperature before use.

② Preparation of reaction working solution：

Before testing, please prepare sufficient reaction working solution according to the test wells. For example, prepare 185 μL of reaction working solution (mix well 130 μL of buffer solution, 35 μL of enzyme co-factor, 10 μL of substrate, 5 μL of enzyme solution and 5 μL of accelerant). Keep reaction working solution on ice protected from light during use. The prepared solution should be used up within 1 day.

③ Preparation of 1 mmol/L standard solution：

Dissolve one vial of standard agent with 1 mL of double distilled water, mix well to dissolve. Keep 1 mmol/L standard solution on ice protected from light during use. The prepared solution should be used up within 1 day.

④ The preparation of standard curve：

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

Dilute 1 mmol/L standard solution with double distilled water to a serial concentration. The recommended dilution gradient is as follows: 0, 0.1, 0.2, 0.25, 0.3, 0.35, 0.4, 0.5 mmol/L. Reference is as follows:

Item	①	②	③	④	⑤	⑥	⑦	⑧
Concentration (mmol/L)	**0**	**0.1**	**0.2**	**0.25**	**0.3**	**0.35**	**0.4**	**0.5**
1 mmol/L standard (μL)	0	20	40	50	60	70	80	100
Double distilled water (μL)	200	180	160	150	140	130	120	100

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 PBS (0.01 M, pH 7.4) with a dounce homogenizer at 4°C.

④ Centrifuge at 10000×g for 10 min at 4°C 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 PBS (0.01 M, pH 7.4) with a ultrasonic

cell disruptor at 4°C.

④ Centrifuge at 10000×g for 10 min at 4°C 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 heart tissue homogenate	1
10% Rat liver tissue homogenate	1
10% Rat kidney tissue homogenate	1
10% Mouse brain tissue homogenate	1
1×10^6 4T1 cells	1
1×10^6 Molt-4 cells	1
1×10^6 Jurkat cells	1

Note: The diluent is PBS (0.01 M, pH 7.4). For the dilution of other sample types, please do pretest to confirm the dilution factor.

The key points of the assay

Keep sample on ice during use. The sample should be tested within 4 hours after processing.

Operating steps

① Standard well: add 20 μL of standards with different concentrations into the standard wells.

Sample well: add 20 μL of sample into the sample wells.

② Add 185 μL of buffer solution to standard well. Add 185 μL of reaction working solution to sample well.

③ Add 20 μL of chromogenic agent to each well.

④ Mix fully for 3 s with microplate reader. Measure the OD values of each well at 450 nm, recorded as A_1. Incubate at room temperature for 20 min. Measure the OD values of each well at 450 nm, recorded as A_2, $\Delta A = A_2 - A_1$. The standard curve is drawn according to A_2.

Calculation

The standard curve:

1. Average the duplicate reading for each standard.

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

3. Plot the standard curve by using absoluted OD 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 SCS in 1 g tissue or cell protein per minute that catalyze the substrate to produce 1 μmol substance at 25℃ is defined as 1 unit.

$$\text{SCS activity (U/gprot)} = (\Delta A_{450} - b) \div a \div T \times 1000 \div C_{pr} \times f$$

[Note]

ΔA_{450}: $\Delta A = A_2 - A_1$

T: The incubation time, 20 min;

1000: 1 mmol/L = 1000 μmol/L

f: Dilution factor of sample before test.

C_{pr}: Concentration of protein in sample, gprot/L.

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)	0.32	0.44	0.67
%CV	8.00	2.50	4.50

Inter-assay Precision

Three rat liver 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)	0.03	0.05	0.14
%CV	4.90	7.60	10.30

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 100%.

	Sample 1	Sample 2	Sample 3
Expected Conc. (mmol/L)	0.31	0.44	0.70
Observed Conc. (mmol/L)	0.32	0.44	0.67
Recovery rate (%)	104.00	100.00	96.00

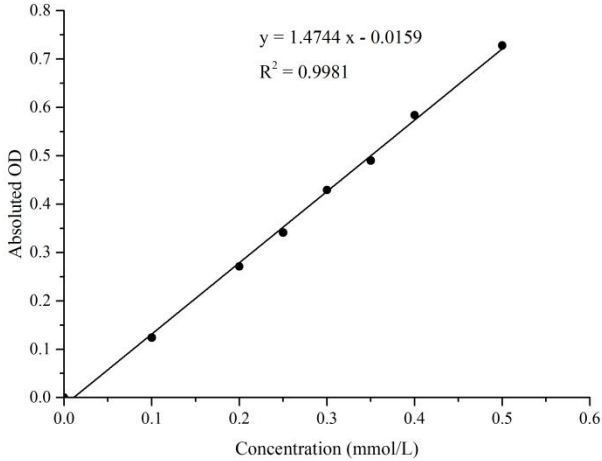
Sensitivity

The analytical sensitivity of the assay is 0.09 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 OD 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 (mmol/L)	0	0.1	0.2	0.25	0.3	0.35	0.4	0.5
OD value	0.062	0.184	0.337	0.407	0.488	0.551	0.641	0.793
	0.059	0.185	0.327	0.396	0.492	0.551	0.649	0.784
Average OD	0.060	0.184	0.332	0.401	0.490	0.551	0.645	0.788
Absoluted OD	0	0.124	0.271	0.341	0.429	0.490	0.584	0.728



Appendix II Example Analysis

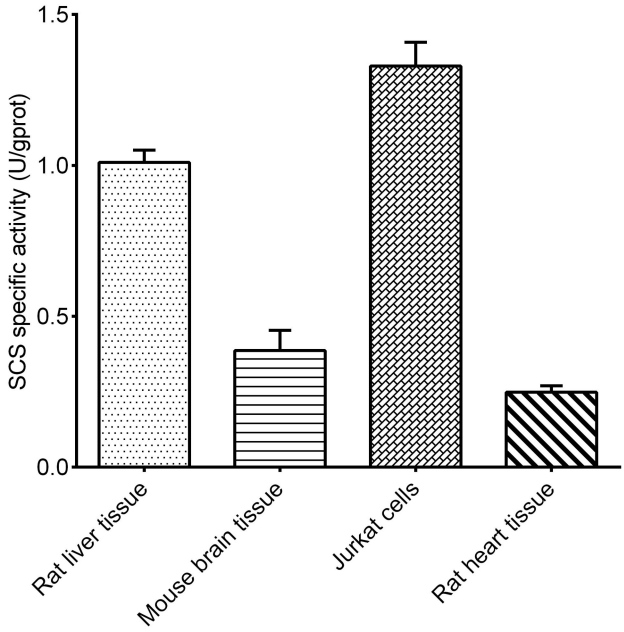
Example analysis:

For 10% rat liver tissue homogenate, take 20 μL of supernatant and carry the assay according to the operation table. The results are as follows:

Standard curve: y = 1.4744 x – 0.0159, the A_1 of the sample is 0.473, the A_2 of the sample is 0.650, $\Delta A_{450} = A_2 - A_1 = 0.650 - 0.473 = 0.177$, the concentration of protein in sample is 6.46 gprot/L, and the calculation result is:

SCS activity (U/gprot) = $(0.177 + 0.0159) \div 1.4744 \div 20 \times 1000 \div 6.46 = 1.012$ U/gprot

Detect 10% rat liver tissue homogenate (the concentration of protein is 6.46 gprot/L), 10% mouse brain tissue homogenate (the concentration of protein is 3.19 gprot/L), 1×10^6 Jurkat cells (the concentration of protein is 0.60 gprot/L) and 10% rat heart tissue homogenate (the concentration of protein is 6.81 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.