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

Catalog No：E-BC-K649-M

Specification：48T(46 samples)/96T(94 samples)

Measuring instrument：Microplate reader（590-610 nm）

Detection range：0.83-65.42 U/L

Elabscience®Succinate Dehydrogenase（SDH）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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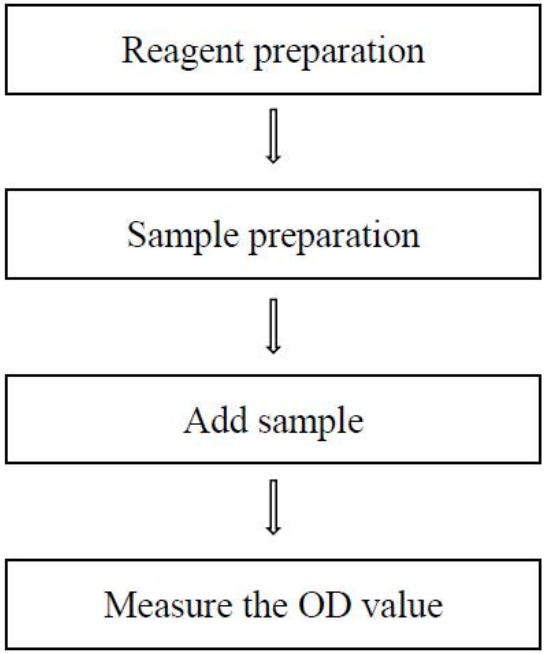
Assay summary



Intended use

This kit can measure succinate dehydrogenase（SDH） activity in animal tissue and cell samples.

Detection principle

SDH catalyzes the dehydrogenation of succinate to fumarate, with electron transport materials transferring electrons to 2, 6-dichlorophenol indiophenol (DCPIP). Then, the reduced DCPIP is reduced to the oxidized DCPIP, which has a characteristic absorption peak at 600 nm. Therefore, the activity of SDH can be quantified by measure the change OD value at 600 nm.

Kit components & storage

Item	Component	Size 1(48 T)	Size 2(96 T)	Storage
Reagent 1	Buffer Solution A	50 mL × 1 vial	50 mL × 2 vials	-20℃, 12 months
Reagent 2	Buffer Solution B	15 mL × 1 vial	30 mL × 1 vial	-20℃, 12 months
Reagent 3	Inhibitor	0.8 mL × 1 vial	0.8 mL × 2 vials	-20℃, 12 months, shading light
Reagent 4	Substrate A	1.2 mL×1 vial	1.2 mL×2 vials	-20℃, 12 months, shading light
Reagent 5	Substrate B	1.2 mL×1 vial	1.2 mL×2 vials	-20℃, 12 months, shading light
Reagent 6	Substrate C	0.6 mL×1 vial	1.2 mL×1 vial	-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 (590-610 nm, optimum wavelength: 600 nm), Centrifuge

Reagents:

Normal saline (0.9% NaCl), PBS(0.01 M，pH 7.4)

Reagent preparation

① Equilibrate all reagents to room temperature before use.

② Preparation of working solution：

For each well，prepare 190 μL of working solution (mix well 140 μL of buffer solution A, 20 μL of substrate A , 20 μL of substrate B and 10 μL of substrate C). The working solution should be prepared on spot and used up in the same day.

Sample preparation

① Sample preparation

Tissue sample:

① Harvest the amount of tissue needed for each assay (initial recommendation 0.1g).

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

③ Homogenize 0.1g tissue in 900 μL buffer solution A and 10 μL inhibitor with a dounce homogenizer at 4℃.

④ Then centrifuge at 600×g for 5 min at 4°C, retaine the supernatant and discard the precipitate. Then centrifuge at 15000×g for 10 min at 4°C, the precipitation is the extracted mitochondria.

⑤ The precipitate was mixed with 200 μL of buffer solution B and 2 μL of inhibitor, sonicated for 5 min at 4°C, centrifuged at 15000×g at 4°C for 10 min. Then take the supernatant for detection.

⑥ 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 2×10^6 cells).

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

③ Homogenize 2×10^6 cells in 400 μL buffer solution A and 4 μL inhibitor with a ultrasonic cell disruptor at 4℃.

④ Centrifuge at 600×g for 5 min at 4°C, retaine the supernatant and discard the precipitate. Centrifuge at 15000×g for 10 min at 4°C, the precipitation is the extracted mitochondria.

⑤ The precipitate was mixed with 200 μL of buffer solution B and 2 μL of inhibitor, sonicated for 5 min at 4°C, centrifuged at 15000×g at 4°C for 10 min. Then take the supernatant for detection.

⑥ 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 heart tissue homogenate	1
10% Rat kidney tissue homogenate	1
10% Mouse liver tissue homogenate	1
10% Mouse heart tissue homogenate	1
10% Mouse kidney tissue homogenate	1

Note: The diluent is buffer solution B. For the dilution of other sample types, please do pretest to confirm the dilution factor.

The key points of the assay

① The supernatant of homogenate sample should be determined on the same day..

② Store some reagents with shading light according to the manual.

③ It is recommended that the number of samples for an experiment be controlled within 8 samples.

Operating steps

① Blank well: Add 20 μL of buffer solution B to the corresponding wells.
Sample well: Add 20 μL of sample to the corresponding wells.

② Add 190 μL of working solution to each well.

③ Mix fully with microplate reader for 5 s and measure the OD value of each well at 600 nm with microplate reader, recorded as A_1. Stand at room temperature for 3 min, measure the OD value of each well at 600 nm with microplate reader, recorded as A_2, $\triangle A = A_1 - A_2$.

Note: It is recommended that the number of samples for an experiment be controlled within 8 samples.

Calculation

The sample:

Tissue and cells sample:

Definition: The amount of SDH in 1 g tissue or cell protein per 1 minute that hydrolyze the substrate to produce 1 μmol product at room temperature is defined as 1 unit.

$$\text{SDH activity (U/gprot)} = \frac{(\Delta A_{sample} - \Delta A_{blank}) \times V_{total} \times f}{(V_{sample} \times 21.8^{*} \times T \times C_{pr})} \times 1000^{*}$$

[Note]

ΔA_{blank}: $A_1 - A_2$.

ΔA_{sample}: $A_1 - A_2$.

f: Dilution factor of sample before test.

V_{total}: The volume of the reaction system, 0.21 mL.

V_{sample}: The volume of the sample, 0.02 mL.

21.8*: Molar absorption coefficient.

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

T: The time of reaction, 3 min.

1000*: 1 mmol/L=1000 μmol/L.

Appendix I Performance Characteristics

1. Parameter:

Intra-assay Precision

Three rat heart tissue 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)	2.60	23.70	52.00
%CV	2.5	1.8	1.7

Inter-assay Precision

Three rat heart tissue 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)	2.60	23.70	52.00
%CV	3.5	4.2	4.3

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 105%.

	Sample 1	Sample 2	Sample 3
Expected Conc. (U/L)	15	32.5	58.5
Observed Conc. (U/L)	15.6	34.1	62.0
Recovery rate (%)	104	105	106

Sensitivity

The analytical sensitivity of the assay is 0.83 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.

Appendix II Example Analysis

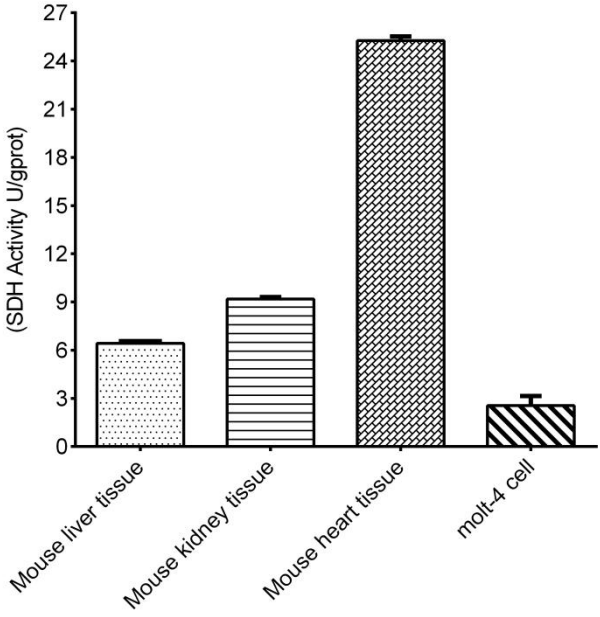
Example analysis：

For mouse kidney tissue, take 20 μL of 10% mouse kidney tissue homogenate, and carry the assay according to the operation steps. The results are as follows:

the OD value of the blank A_1 is 1.358, the OD value of the blank A_2 is 1.355,the OD value of the sample A_1 is 1.000, the OD value of the sample A_2 is 0.382, the concentration of protein in sample is 11.20 gprot/L, and the calculation result is:

$$\text{SDH activity (U/gprot)} = \frac{(1.000-0.382) - (1.358 - 1.355) \times 0.21}{0.02 \times 21.8 \times 3 \times 11.20} \times 1000 = 8.82 \text{ U/gprot}$$

Detect 10% mouse liver tissue homogenate (the concentration of protein is 12.51 gprot/L), 10% mouse kidney tissue homogenate (the concentration of protein is 11.20 gprot/L), 10% mouse heart tissue homogenate (the concentration of protein is 3.10 gprot/L) and Molt-4 cell (the concentration of protein is 0.79 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.