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

Catalog No：E-BC-K902-M

Specification：96T(40 samples)

Measuring instrument：Microplate reader(550-560 nm)

Detection range：0.043-0.5 mmol/L

Elabscience® Succinic Acid Colorimetric 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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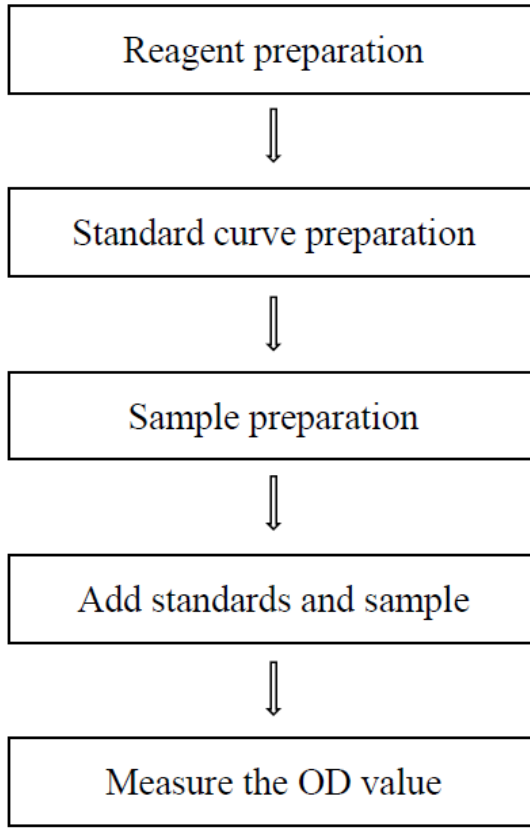
Assay summary



Intended use

This kit can measure succinic acid content in serum, plasma, animal tissue and cell samples.

Detection principle

Succinic acid, is widely present in all plant and animal tissues, was first extracted from amber, is an intermediate in the citric acid cycle, and plays an important role in the production of energy in cells. Succinate, or succinate, is widely used in the agricultural, food and pharmaceutical industries due to its low toxicity.

Succinic acid is catalyzed by enzyme reagent to form colored substance with color developer. The maximum absorption is at 555 nm. The content of succinic acid in samples can be calculated by measuring OD value and standard curve at 555 nm.

Kit components & storage

Item	Component	Size (96 T)	Storage
Reagent 1	Buffer Solution A	50 mL ×1 vial	-20°C, 12 months
Reagent 2	Buffer Solution B	50 mL ×1 vial	-20°C, 12 months
Reagent 3	Substrate	Powder ×2 vials	-20°C, 12 months, shading light
Reagent 4	Accelerant	Powder ×2 vials	-20°C, 12 months, shading light
Reagent 5	Enzyme Reagent A	0.5 mL ×1 vial	-20°C, 12 months, shading light
Reagent 6	Enzyme Reagent B	1.5 mL ×1 vial	-20°C, 12 months, shading light
Reagent 7	Chromogenic Agent	5 mL ×1 vial	-20°C, 12 months, shading light
Reagent 8	4 mmol/L Standard Solution	1 mL ×1 vial	-20°C, 12 months, shading light
	Microplate	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 (550-560 nm, optimum wavelength: 555 nm)，Incubator(37℃)

Reagents:

PBS (0.01 M, pH 7.4)

Consumptive material:

10kDa MWCO Spin Filter

Reagent preparation

① Equilibrate all reagents to 25℃ before use.

② The preparation of substrate working solution：
Dissolve one vial of substrate with 0.5 mL of buffer solution A, mix well to dissolve and keep it on ice for detection. Store at -20 ℃ for 2 days protected from light.

③ The preparation of accelerant working solution：
Dissolve one vial of accelerant with 1.5 mL of buffer solution B, mix well to dissolve and keep it on ice for detection. Store at -20 ℃ for 2 days protected from light.

④ The preparation of enzyme reagent A working solution：
Before testing, please prepare sufficient enzyme reagent A working solution according to the test wells. For example, prepare 50 μL of enzyme reagent A working solution (mix well 45 μL of buffer solution A and 5 μL of enzyme reagent A). The enzyme reagent A working solution should be kept on ice

protected from light and used up within 1 day.

⑤ The preparation of measuring working solution:

Before testing, please prepare sufficient measuring working solution according to the test wells. For example, prepare 150 μL of measuring working solution (mix well 140 μL of buffer solution A and 10 μL of substrate working solution). The measuring working solution should be kept on ice protected from light and used up within 1 day.

⑥ The preparation of chromogenic working solution:

Before testing, please prepare sufficient chromogenic working solution according to the test wells. For example, prepare 180 μL of chromogenic working solution (mix well 130 μL of buffer solution B, 10 μL of accelerant working solution, 10 μL of enzyme reagent B and 30 μL of chromogenic agent). It is recommended to prepare chromogenic working solution after adding the measuring working solution to the wells and keep it on ice protected from light and used up within 30 min.

⑦ The preparation of 0.5 mmol/L standard solution:

Before testing, please prepare sufficient 0.5 mmol/L standard solution according to the test wells. For example, prepare 960 μL of 0.5 mmol/L standard solution (mix well 840 μL of double distilled water and 120 μL of 4 mmol/L standard solution). The 0.5 mmol/L standard solution should be prepared on spot protected from light and used up within 1 day.

⑧ The preparation of standard curve:

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

Dilute 0.5 mmol/L standard solution with double distilled water to a serial concentration. The recommended dilution gradient is as follows: 0, 0.10, 0.15, 0.20, 0.30,0.35, 0.40, 0.50 mmol/L. Reference is as follows:

Item	①	②	③	④	⑤	⑥	⑦	⑧
Concentration (mmol/L)	**0**	**0.10**	**0.15**	**0.20**	**0.30**	**0.35**	**0.40**	**0.50**
0.5 mmol/L Standard (μL)	0	40	60	80	120	140	160	200
Double distilled water (μL)	200	160	140	120	80	60	40	0

Sample preparation

① Sample preparation

Serum and plasma: Put sample into 10kDa MWCO Spin Filter and centrifuge at 12000×g for 15 min. Collect the filtrate and preserve it on ice for detection.

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 double distilled water with a dounce homogenizer at 4 ℃.

④ Centrifuge at 10000×g for 10 min at 4℃ to remove insoluble material.

⑤ Collect 100-500 μL of supernatant and add it to 10kDa MWCO Spin Filter. Centrifuge at 12000×g for 15 min at 4℃.

⑥ Take the filtered sample supernatant and preserve it on ice for detection.

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 normal saline (0.9% NaCl).

③ Homogenize 1×10^6 cells in 200 μL double distilled water with a dounce homogenizer at 4 ℃.

④ Centrifuge at 10000×g for 10 min at 4℃ to remove insoluble material.

⑤ Collect 100-500 μL of supernatant and add it to 10kDa MWCO Spin Filter. Centrifuge at 12000×g for 15 min at 4℃.

⑥ Take the filtered sample supernatant and preserve it on ice for detection.

② Dilution of sample

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

Sample type	Dilution factor
10% Mouse liver tissue homogenate	**1**
10% Mouse kidney tissue homogenate	**1**
10% Rats liver tissue homogenate	**1**
10% Rats heart tissue homogenate	**1**
10% Rats lung tissue homogenate	**1**
Human serum	**1**
1×10^6 Molt-4 cells	**1**
1×10^6 HL-60 cells	**1**
1×10^6 Jurkat cells	**1**
1×10^6 Hela cells	**1**

Note: The diluent is double distilled water. For the dilution of other sample types, please do pretest to confirm the dilution factor.

Operating steps

① Standard well: Add 20 μL of standard solution with different concentrations into the corresponding wells.

Sample well: Add 20 μL of sample into sample wells.

Control well: Add 20 μL of sample into control wells.

② Add 30 μL of enzyme reagent A working solution into standard and sample wells.

Add 30 μL of buffer solution A into control wells.

③ Add 80 μL of measuring working solution into each well.

④ Mix fully for 5 s with microplate reader. Incubate at 37 ℃ for 5 min protected from light.

⑤ Add 120 μL of chromogenic working solution into each well.

⑥ Mix fully for 5 s with microplate reader. Incubate at 37 ℃ for 15 min protected from light. Measure the OD values of each well at 555 nm with microplate reader.

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:

1. Serum and plasma samples:

$$\text{succinic acid content (mmol/L)} = (\Delta A_{555} - b) \div a \times f$$

2. Tissue sample:

$$\text{succinic acid content (mmol/kg wet weight)} = (\Delta A_{555} - b) \div a \div \frac{m}{V} \times f$$

3. Cell sample:

$$\text{succinic acid content (μmol/10^6)} = (\Delta A_{555} - b) \div a \div \frac{n}{V} \times f$$

[Note]

ΔA_{555}: $\Delta A_{555} = A_{Sample} - A_{Control}$.

f: Dilution factor of sample before test.

m: The weight of tissue, g.

V: The volume of normal saline in the preparation step of sample, mL.

n: The number of cell sample/10^6.

Appendix I Performance Characteristics

1. Parameter:

Intra-assay Precision

Three human serum 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 (mmol/L)	0.20	0.30	0.40
%CV	4.0	6.0	3.0

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 (mmol/L)	0.20	0.30	0.40
%CV	5.1	9.3	3.4

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 93%.

	Standard 1	Standard 2	Standard 3
Expected Conc.(mmol/L)	0.20	0.30	0.40
Observed Conc.(mmol/L)	0.190	0.27	0.37
recovery rate(%)	95	90	93

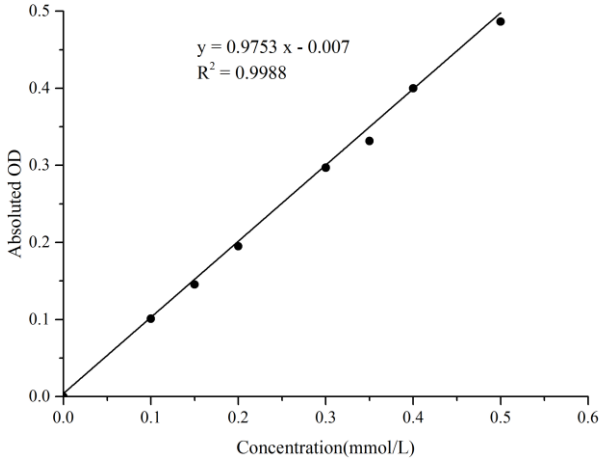
Sensitivity

The analytical sensitivity of the assay is 0.043 mmol/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**	**0.10**	**0.15**	**0.20**	**0.30**	**0.35**	**0.40**	**0.50**
OD Value	0.405	0.496	0.548	0.598	0.684	0.733	0.797	0.884
	0.390	0.501	0.538	0.587	0.705	0.725	0.798	0.884
Average OD	0.398	0.499	0.543	0.593	0.695	0.729	0.798	0.884
Absoluted OD	0.000	0.101	0.146	0.195	0.297	0.332	0.400	0.487



Appendix II Example Analysis

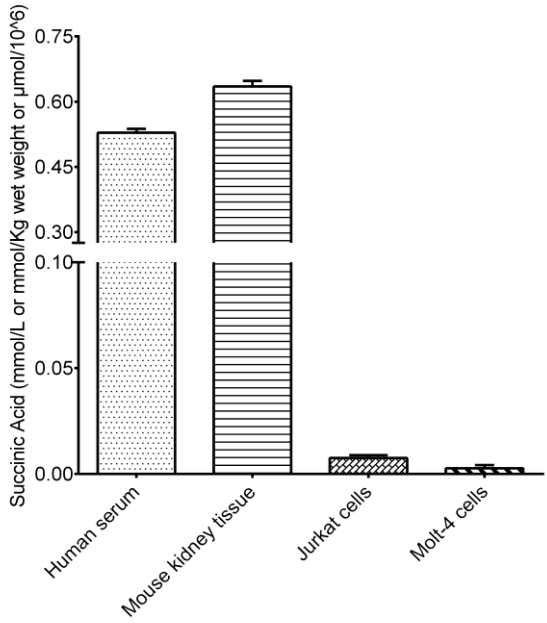
Example analysis:

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

Standard curve: y = 0.9753 x - 0.007, the OD value of the sample is 0.622, the OD value of the control is 0.574, $\Delta A_{555} = A_{Sample} - A_{control} = 0.622 - 0.574 = 0.048$, and the calculation result is:

$$\underset{\text{(mmol/kg wet weight)}}{\text{succinic acid content}} = (0.048 + 0.007) \div 0.9753 \div \frac{0.1}{0.9} \times 1 = 0.627 \text{ mmol/kg wet weight}$$

Detect human serum, 10% mouse kidney tissue homogenate, 1.12×10^6 Jurkat cells and 2.13×10^6 Molt-4 cells, 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.