TECHNICAL MANUAL

Succinic Acid Colorimetric Assay Kit

- **SKU CODE:** MAES0489
- **SIZE:** 96 Tests
- **DETECTION PRINCIPLE:** Assay Kit
- **RUO:** Research-Use-Only

Revised: 01/2026

1. Assay summary

- Reagent preparation
- Standard curve preparation
- Sample preparation
- Add standards and samples
- Enzyme reaction
- Chromogenic reaction
- Measure absorbance

2. Intended use

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

3. Detection principle

Succinic acid is widely present in all plant and animal tissues. It was first extracted from amber and is an intermediate in the citric acid cycle, playing an important role in cellular energy production. 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 a 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.

4. 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

Item	Component	Size (96 T)	Storage
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	

5. Materials prepared by users

Instruments:

Microplate reader (550-560 nm, optimum wavelength: 555 nm), Incubator (37°C)

Reagents:

PBS (0.01 M, pH 7.4)

Consumptive material:

10kDa MWCO Spin Filter

6. Reagent preparation

1. Equilibrate all reagents to 25°C before use.
2. **Preparation of substrate working solution:** Dissolve one vial of substrate with 1 mL of buffer solution A, mix well to dissolve and keep it on ice for detection. Store at -20°C for 2 days protected from light.
3. **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°C for 2 days protected from light.
4. **Preparation of enzyme reagent A working solution:** Before testing, 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. **Preparation of measuring working solution:** Before testing, 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. **Preparation of chromogenic working solution:** Before testing, 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. **Preparation of 0.5 mmol/L standard solution:** Before testing, 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. **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.

7. Sample preparation

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°C.

4. Centrifuge at 10000×g for 10 min at 4°C 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°C.

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°C.

4. Centrifuge at 10000×g for 10 min at 4°C 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°C.

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

Dilution of sample

The recommended dilution factor for different samples is as follows (for reference only): All listed samples use dilution factor 1. The diluent is double distilled water. For the dilution of other sample types, please do pretest to confirm the dilution factor.

8. 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°C 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°C for 20 min protected from light. Measure the OD values of each well at 555 nm with microplate reader.

9. 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 absolute OD value.

3. Plot the standard curve by using absolute 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:
   succinic acid content (mmol/L) = (ΔA_555 - b) ÷ a × f
2. Tissue sample:
   succinic acid content (mmol/kg wet weight) = (ΔA_555 - b) ÷ a ÷ m/V × f
3. Cell sample:
   succinic acid content (μmol/10^6) = (ΔA_555 - b) ÷ a ÷ n/V × f

[Note]

ΔA_555: ΔA_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.

10. Appendix I Performance Characteristics

Parameter:

Intra-assay Precision

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

Intra-assay Precision

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.

Inter-assay Precision

Parameters	Sample 1	Sample 2	Sample 3
Mean (mmol/L)	0.20	0.30	0.40
%CV	5.1	9.3	3.4

Recovery

Three samples of high concentration, middle concentration and low concentration were tested with each concentration measured 6 times in parallel to get the average recovery rate of 93%.

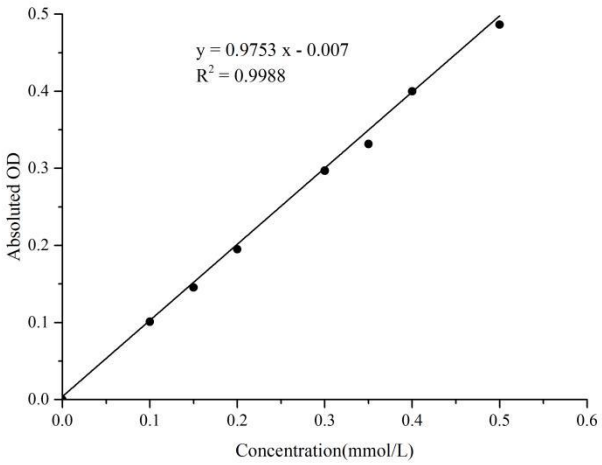
	Standard 1	Standard 2	Standard 3
Expected Conc.(mmol/L)	0.20	0.30	0.40
Observed Conc.(mmol/L)	0.190	0.27	0.37
Recovery rate(%)	95	90	93

Sensitivity

The analytical sensitivity of the assay is 0.007 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.

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), the standard curve and data are provided 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
Absolute OD	0.000	0.101	0.146	0.195	0.297	0.332	0.400	0.487

11. Appendix II Example Analysis

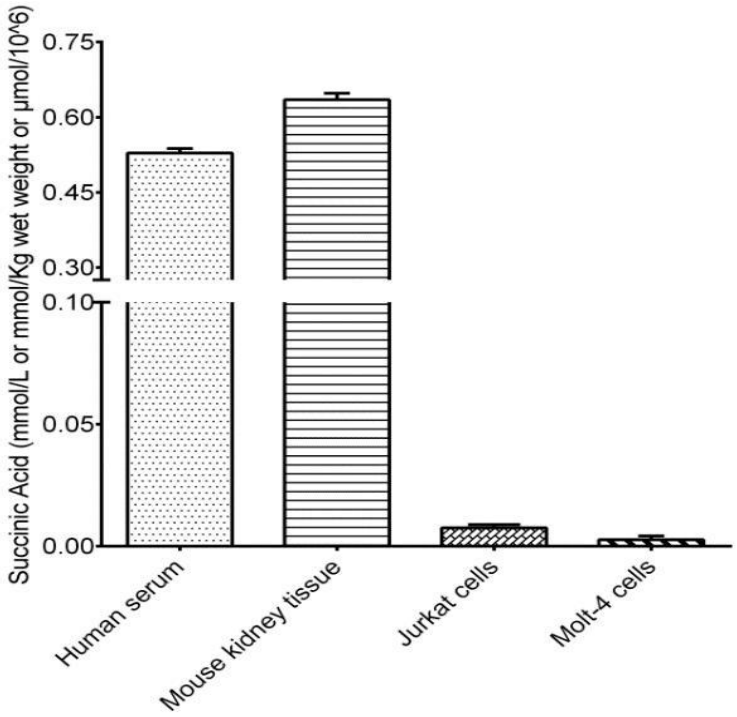
Example analysis:

Take 20 µL of 10% mouse kidney tissue homogenate and carry out 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:

succinic acid content (mmol/kg wet weight) = (0.048 + 0.007) ÷ 0.9753 ÷ 0.1/0.9 × 1 = 0.627 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:



12. Statement

1. This assay kit is for Research Use Only. Assay Genie assumes no responsibility for any problems or legal liabilities arising from the use of this kit for clinical diagnosis or any other purpose.
2. Please read the instructions carefully and calibrate the instruments before performing the experiments. Follow the instructions strictly throughout the procedure.
3. Appropriate protective measures must be taken, including wearing a lab coat and latex gloves.
4. If the concentration of the substance falls outside the detection range, perform an additional dilution or concentration step on the sample.
5. It is recommended to perform a pre-test if your sample type is not listed in the instruction manual.
6. Experimental results are closely related to reagent quality, operator technique, environmental conditions, and other factors. Assay Genie guarantees the quality of the kits only and is NOT responsible for sample consumption resulting from use of the assay kits. It is advisable to estimate the expected sample usage and reserve sufficient samples before starting the experiment.

Assay Genie 100% money-back guarantee!

If you are not satisfied with the quality of our products and our technical team cannot resolve your problem, we will give you 100% of your money back.

Manufacturers Statement: This final kit system is assembled and quality-released by Assay Genie Limited.