



## **TECHNICAL MANUAL**

### **Citrate Synthase (CS) Activity Assay Kit**

- **SKU CODE:** MAES0239
- **SIZE:** 48 Tests/96 Tests
- **DETECTION PRINCIPLE:** Assay Kit
- **RUO:** Research-Use-Only

## 1. Assay summary

- Reagent preparation
- Standard curve preparation
- Sample preparation
- Add buffer solution and substrate
- Add chromogenic agent and mix
- Add standards and samples
- Measure absorbance values

## 2. Intended use

This kit can measure citrate synthase (CS) activity in animal tissue, plant tissue, and cell samples.

## 3. Detection principle

Citrate synthase (CS) catalyzes the reaction between acetyl-CoA and oxaloacetate to produce citryl-CoA, which can further produce CoA. The yellow product generated by this reaction has a characteristic absorption peak at 412 nm. The activity of CS can be calculated by measuring the change in absorbance value at 412 nm.

## 4. Kit components & storage

Item	Component	Size 1(48 T)	Size 2(96 T)	Storage
Reagent 1	Extraction Solution	50 mL × 1 vial	50 mL × 2 vials	-20°C, 12 months
Reagent 2	Buffer Solution	9 mL × 1 vial	18 mL × 1 vial	-20°C, 12 months
Reagent 3	Substrate	1.8 mL × 1 vial	1.8 mL × 2 vials	-20°C, 12 months, shading light
Reagent 4	Chromogenic Agent	1.6 mL × 1 vial	1.6 mL × 2 vials	-20°C, 12 months, shading light
Reagent 5	Standard	Powder × 1 vial	Powder × 2 vials	-20°C, 12 months, shading light
	Microplate	48 wells	96 wells	No requirement

Item	Component	Size 1(48 T)	Size 2(96 T)	Storage
	Plate Sealer	2 pieces	2 pieces	

## 5. Materials prepared by users

**Instruments:** Incubator, centrifuge, microplate reader (402-422 nm, optimum wavelength: 412 nm)

**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 to ensure sufficient amount of reagents.

## 6. Reagent preparation

After melting, keep the chromogenic agent on ice for use but no more than 2 hours, and return it immediately to -20°C after use. Equilibrate all reagents to room temperature before use.

2. Preparation of 10 mmol/L standard solution: Dissolve one vial of standard with 1 mL double-distilled water and mix thoroughly. Aliquot the 10 mmol/L standard solution and store at -20°C for 3 days protected from light. Avoid repeated freeze/thaw cycles.

3. Preparation of 1 mmol/L standard solution: Prepare 1000 µL of 1 mmol/L standard solution by mixing 100 µL of 10 mmol/L standard solution with 900 µL of double-distilled water. The 1 mmol/L standard solution should be prepared fresh and used within 2 hours.

4. 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 serial concentrations. The recommended dilution gradient is as follows: 0, 0.2, 0.3, 0.4, 0.6, 0.8, 0.9, 1 mmol/L.

## 7. Sample preparation

**Sample preparation:**

**Tissue sample:**

1. Harvest the amount of tissue needed for each assay (initial recommendation 20 mg).
2. Homogenize 20 mg tissue in 180 µL extracting solution with a dounce homogenizer at 4°C.

3. Centrifuge at 10,000×g for 15 minutes to remove insoluble material. Collect supernatant and keep it on ice for detection.
4. Meanwhile, determine the protein concentration of supernatant (MAES0177).

**Cells:**

1. Harvest the number of cells needed for each assay (initial recommendation 1×10<sup>6</sup> cells).
2. Wash cells with PBS (0.01 M, pH 7.4).
3. Homogenize 1×10<sup>6</sup> cells in 200 μL extracting solution with an ultrasonic cell disruptor at 4°C.
4. Centrifuge at 10,000×g for 10 minutes to remove insoluble material. Collect supernatant and keep it on ice for detection.
5. Meanwhile, determine the protein concentration of supernatant (MAES0177).

**Dilution of sample:**

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

- 10% Rat kidney tissue homogenate: 1
- 10% Rat spleen tissue homogenate: 1
- 10% Rat liver tissue homogenate: 1
- 10% Rat heart tissue homogenate: 1
- 10% Mouse liver tissue homogenate: 1
- 10% Mouse lung tissue homogenate: 1

**Note:** The diluent is extracting solution. For the dilution of other sample types, please perform a pretest to confirm the dilution factor.

## 8. The key points of the assay

1. Avoid bubbles when adding reagents.
2. After melting, keep reagent 4 on ice for use but no more than 2 hours, and return it immediately to -20°C after use.

## 9. Operating steps

1. Add 125 μL of buffer solution to standard wells and sample wells.
2. Add 30 μL of substrate to each well.
3. Add 20 μL of chromogenic agent to each well.
4. Mix thoroughly with microplate reader for 3 seconds, then incubate at 37°C for 3 minutes.

5. Standard wells: Add 10  $\mu\text{L}$  of standard solution with different concentrations to the corresponding wells. Sample wells: Add 10  $\mu\text{L}$  of sample to the corresponding wells.
6. Mix thoroughly with microplate reader for 3 seconds and measure the OD value of each well at 412 nm with microplate reader. Record as A1.
7. Incubate at 37°C for 8 minutes.
8. Mix thoroughly with microplate reader for 3 seconds and measure the OD value of each well at 412 nm with microplate reader. Record as A2. Calculate  $\Delta A_{412} = A2 - A1$ . (Plot the standard curve using A2 values).

## 10. 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 using absolute OD value of standard and corresponding concentration as y-axis and x-axis respectively. Create the standard curve ( $y = ax + b$ ) with graph software (or Excel).

### The sample:

Tissue sample and cell sample:

**Definition:** The amount of CS in 1 g tissue or cell protein per 1 minute that produces 1  $\mu\text{mol}$  CoA at 37°C is defined as 1 unit.

$$\text{CS activity (U/g}_{\text{prot}}) = (\Delta A_{412} - b) \div a \div T \times 1000 \div C_{\text{pr}} \times f$$

[Note]

$\Delta A_{412}$ : The change in OD value of sample well.

T: The time of incubation reaction, 8 min

1000: 1 mmol/L = 1000  $\mu\text{mol/L}$ .

$C_{\text{pr}}$ : The concentration of protein in sample,  $\text{g}_{\text{prot}}/\text{L}$ .

f: Dilution factor of sample before test.

## 11. Appendix

### Performance Characteristics

#### Intra-assay Precision

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

Three mouse liver tissue samples were assayed 17 times in duplicate by three operators to determine precision between assays.

#### Recovery

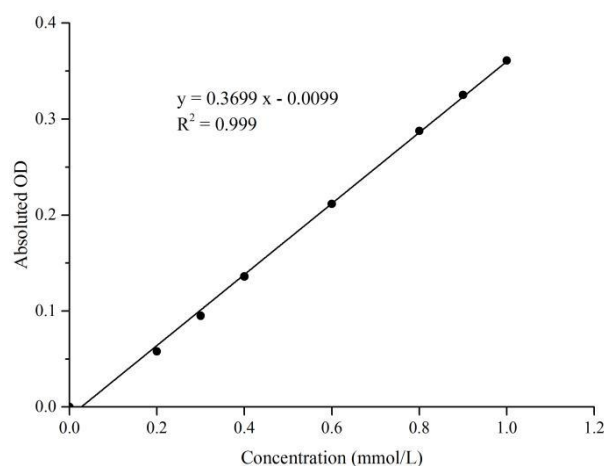
Three samples of high concentration, middle concentration and low concentration were tested with each concentration measured 6 times in parallel to obtain an average recovery rate of 104%.

#### Sensitivity

The analytical sensitivity of the assay is 2.09 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.

#### Standard curve data

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:



## Example Analysis

For mouse liver tissue, take 10  $\mu\text{L}$  of 10% mouse liver tissue homogenate, and carry out the assay according to the operation table. The results are as follows:

**Standard curve:**  $y = 0.3699x - 0.0099$

The OD value of the sample ( $A_1$ ) is 0.695

The OD value of the sample ( $A_2$ ) is 0.871

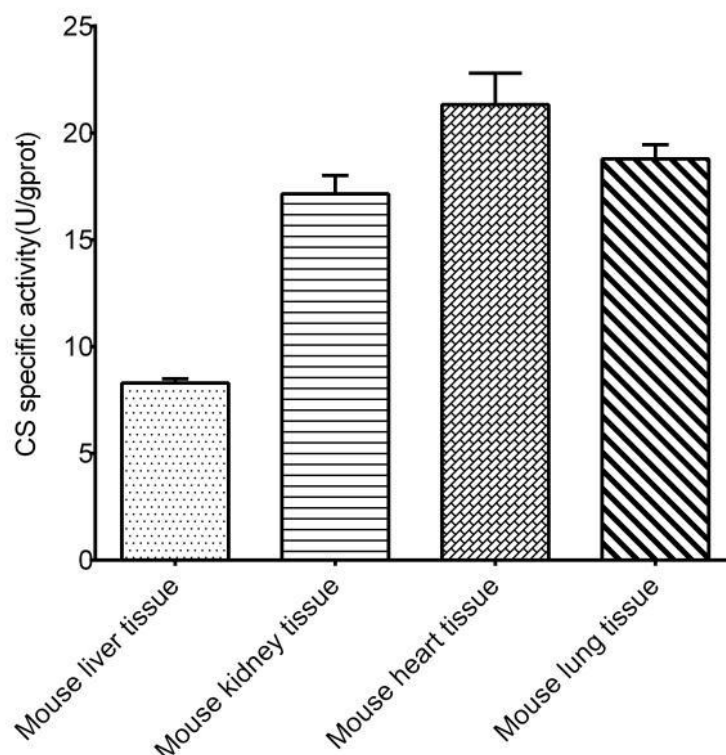
$$\Delta A_{412} = A_2 - A_1 = 0.871 - 0.695 = 0.176$$

The concentration of protein in sample is 7.40  $\text{g}_{\text{prot}}/\text{L}$

The calculation result is:

$$\text{CS activity (U/g}_{\text{prot}}) = (0.871 - 0.695 + 0.0099) \div 0.3699 \div 8 \times 1000 \div 7.40 = 8.49 \text{ U/g}_{\text{prot}}$$

Detection of 10% mouse liver tissue homogenate (protein concentration: 7.40  $\text{g}_{\text{prot}}/\text{L}$ ), 10% mouse kidney tissue homogenate (protein concentration: 5.36  $\text{g}_{\text{prot}}/\text{L}$ ), 10% mouse heart tissue homogenate (protein concentration: 3.73  $\text{g}_{\text{prot}}/\text{L}$ ), and 10% mouse lung tissue homogenate (protein concentration: 2.57  $\text{g}_{\text{prot}}/\text{L}$ ) according to the protocol. The results are 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.

**Note:**

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**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.**