TECHNICAL MANUAL

Citric Acid (CA) Colorimetric Assay Kit

- **SKU CODE:** MAES0179
- **SIZE:** 48 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
- Measure the absorbance values

2. Intended use

This kit can be used to measure citric acid (CA) content in animal tissue, serum (plasma), and mitochondria samples.

3. Detection principle

Under acidic conditions, Cr(VI) is reduced to Cr^{3+}, which reacts with citric acid. The resulting product has a characteristic absorption peak at 545 nm, allowing the citric acid content in the sample to be calculated by measuring the absorbance value at 545 nm.

4. Kit components & storage

Item	Component	Size 1(48 T)	Size 2(96 T)	Storage
Reagent 1	Extracting Solution	60 mL x 1 vial	60 mL x 2 vials	2-8 °C, 12 months
Reagent 2	Lysis Buffer	10 mL x 1 vial	20 mL x 1 vial	2-8 °C, 12 months
Reagent 3	Reducing Agent	Powder x 1 vial	Powder x 1 vial	2-8 °C, 12 months, shading light
Reagent 4	Chromogenic Agent	1.5 mL x 1 vial	1.5 mL x 2 vials	2-8 °C, 12 months, shading light
Reagent 5	2 mmol/L CA Standard	2 mL x 1 vial	2 mL x 1 vial	2-8 °C, 12 months
	Microplate	48 wells	96 wells	No requirement
	Plate Sealer	2 pieces	2 pieces	

5. Materials prepared by users

Instruments:

Microplate reader (535-555 nm), Micropipettor, Water bath, Incubator, Vortex mixer, Centrifuge

Reagents:

Double distilled water

6. Reagent preparation

1. Equilibrate all reagents to room temperature before use.
2. If there are solids in the buffer solution, heat at 80°C until the solution becomes clear, then cool before use.
3. **Preparation of reducing agent working solution:** Dissolve one vial of reducing agent with 5 mL of double distilled water and mix thoroughly. Store in aliquots at -20°C for up to 7 days, protected from light.
4. **Preparation of standard curve:** Always prepare a fresh set of standards. Discard working standard dilutions after use. Dilute the 2 mmol/L standard with double distilled water to create a serial concentration. The recommended dilution gradient is: 2.0, 1.5, 1.2, 1.0, 0.8, 0.5, 0.2, 0 mmol/L. Standard dilution table: Concentration (mmol/L): 0, 0.2, 0.5, 0.8, 1.0, 1.2, 1.5, 2.0 2 mmol/L standard (µL): 0, 20, 50, 80, 100, 120, 150, 200 Double distilled water (µL): 200, 180, 150, 120, 100, 80, 50, 0

7. Sample preparation

1. **Sample preparation:** Extraction of citric acid in liquid samples: Detect directly. Extraction of citric acid in tissue samples: 1. Take 0.1 g tissue, add 0.9 mL of extracting solution, then homogenize the sample in an ice water bath. 2. Centrifuge at 11,000 x g for 10 min at 4°C, then take the supernatant and keep on ice for measurement. Extraction of citric acid in mitochondria: 1. Take 0.1 g tissue, add 0.9 mL of extracting solution, then homogenize the sample in an ice water bath. 2. Centrifuge at 600 x g for 5 min at 4°C, then transfer the supernatant to another EP tube and centrifuge at 10,000 x g for 10 min at 4°C. Discard the supernatant (this supernatant can be used for determining cytoplasmic citric acid content). 3. Add 200 µL of lysis buffer and dissolve completely using a vortex mixer. 4. Centrifuge at 10,000 x g for 10 min at 4°C, then take the supernatant and

keep on ice for measurement. 5. Meanwhile, determine the protein concentration of the supernatant (MAES0177).

2. **Sample dilution:** The recommended dilution factors for different samples are as follows (for reference only): Human serum: 3-15 Dog serum: 3-10 Rat serum: 3-15 Horse serum: 3-10 Mouse plasma: 3-10 10% Rat brain tissue homogenate: 5-10 10% Rat liver tissue homogenate: 5-20 10% Rat kidney tissue homogenate: 5-10 10% Rat lung tissue homogenate: 15-30 10% Mouse heart tissue homogenate: 5-20 Note: The diluent is extracting solution. For other sample types, perform a pretest to confirm the dilution factor.

8. The key points of the assay

Ensure there are no bubbles in the microplate wells when measuring the OD value.

9. Operating steps

1. Standard tubes: Add 30 µL of standard solution with different concentrations into 1.5 mL EP tubes. Sample tubes: Add 30 µL of sample into 1.5 mL EP tubes.
2. Add 210 µL of extracting solution to each tube.
3. Add 30 µL of reducing agent working solution to each tube.
4. Add 30 µL of chromogenic agent to each tube.
5. Mix thoroughly and incubate at room temperature for 30 min. Transfer 200 µL of supernatant to the microplate. Measure the OD values of each well at 545 nm using a microplate reader.

10. Calculation

The standard curve:

1. Average the duplicate readings 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 the absolute OD value of standards as the y-axis and corresponding concentrations as the x-axis. Create the standard curve (y = ax + b) using graph software (or Excel).

The sample:

1. Serum (plasma) sample:

CA content (mmol/L) = (ΔA - b) ÷ a × f

2. Tissue sample:

CA content (μmol/g wet weight) = (ΔA - b) ÷ a × f ÷ m × V

3. Mitochondria sample:

CA content (mmol/g_{prot}) = (ΔA - b) ÷ a ÷ C_{pr} × f

[Note]

ΔA: OD_{Sample} - OD_{Blank}

f: Dilution factor of sample before test

m: The weight of tissue sample (0.1 g)

V: The volume of extracting solution (0.9 mL)

C_{pr}: Protein concentration of sample (g_{prot}/L)

11. Appendix

Performance Characteristics

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)	0.55	1.02	1.80
%CV	4.3	3.9	3.8

Inter-assay Precision

Three human serum samples were assayed 17 times in duplicate by three operators to determine precision between assays.

Inter-assay Precision

Parameters	Sample 1	Sample 2	Sample 3
Mean (mmol/L)	0.55	1.02	1.80
%CV	3.9	4.2	3.9

Recovery

Three samples of high, middle, and low concentrations were tested with each concentration measured 6 times in parallel to obtain an average recovery rate of 95%.

Recovery

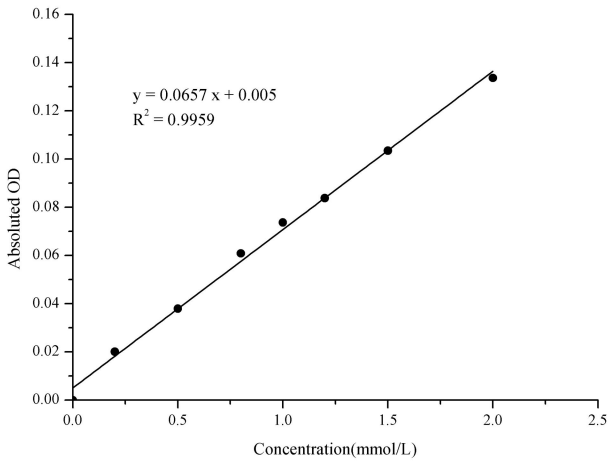
Standard	Expected Conc. (mmol/L)	Observed Conc. (mmol/L)	Recovery rate (%)
Standard 1	0.45	0.4	96
Standard 2	0.85	0.8	94
Standard 3	1.3	1.2	95

Sensitivity

The analytical sensitivity of the assay is 0.06 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 data

Since the OD values of the standard curve may vary according to actual assay performance conditions (e.g., operator, pipetting technique, or temperature effects), the standard curve and data provided below are for reference only:



Standard curve data

Concentration (mmol/L)	OD value 1	OD value 2	Average OD	Absolute OD
0.0	0.071	0.071	0.071	0.000
0.2	0.091	0.091	0.091	0.020
0.5	0.112	0.106	0.109	0.038
0.8	0.136	0.127	0.132	0.061
1.0	0.147	0.142	0.145	0.074
1.2	0.154	0.156	0.155	0.084
1.5	0.177	0.172	0.174	0.103
2.0	0.204	0.205	0.205	0.134

12. Example Analysis

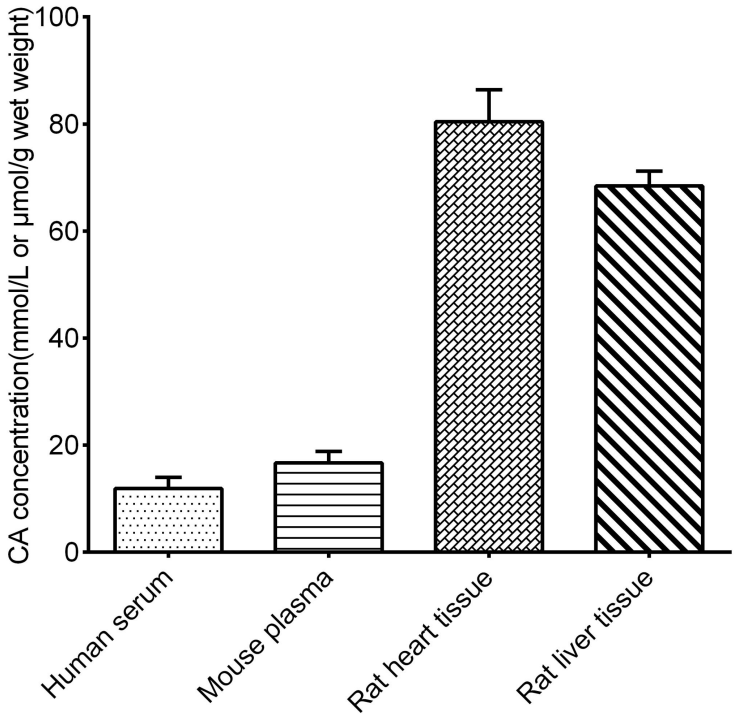
Example analysis:

Take 0.1 mL of human serum and add 0.9 mL of extracting solution, mix thoroughly and extract by vortexing for 1 min. Take 30 µL for the corresponding tubes and perform the assay according to the operating steps. The results are as follows:

Standard curve: y = 0.0672x + 0.0009, the OD value of the sample is 0.142, the OD value of the blank is 0.071, and the calculation result is:

CA content (mmol/L) = (0.142 - 0.071 - 0.0009) ÷ 0.0672 × 10 = 10.432 mmol/L

Detection of human serum (diluted 10 times), mouse plasma (diluted 10 times), 10% rat heart tissue homogenate (diluted 20 times), and 10% rat liver tissue homogenate (diluted 20 times) according to the protocol yielded the following results:



13. 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.