TECHNICAL MANUAL

Calcium (Ca) Colorimetric Assay Kit

- **SKU CODE:** MAES0091
- **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 sample
- Measure the OD value at 610 nm

2. Intended use

This kit is used for the determination of calcium content in serum, plasma, urine and tissue samples.

3. Detection principle

Calcium ions in the sample bind to Methyl Thymol Blue (MTB) in alkaline solution and form a blue complex. Calcium content can be calculated by measuring the OD value at 610 nm.

4. Kit components & storage

Item	Component	Size 1 (48 T)	Size 2 (96 T)	Size 3 (500Assays)	Storage
Reagent 1	MTB Reagent	5 mL x 1 vial	10 mL x 1 vial	10 mL x 5 vials	2-8 °C, 12 months, protect from light
Reagent 2	Alkali Reagent	10 mL x 1 vial	20 mL x 1 vial	50 mL x 2 vials	2-8 °C, 12 months
Reagent 3	Clarificant	1 mL x 1 vial	1 mL x 1 vial	5 mL x 1 vial	2-8 °C, 12 months
Reagent 4	2.5 mmol/L Calcium Standard	5 mL x 1 vial	10 mL x 1 vial	50 mL x 1 vial	2-8 °C, 12 months
	Microplate	48 wells	96 wells	/	No requirement
	Plate Sealer	2 pieces	2 pieces	2 pieces	

5. Materials prepared by users

Instruments:

Microplate reader (600-620 nm, optimum wavelength: 610 nm), Micropipette, Centrifuge, Incubator, Vortex mixer

Reagents:

Deionized water

6. Reagent preparation

1. Equilibrate all reagents to room temperature before use.
2. The clarificant will be solid at 2-8°C. Preheat the clarificant at 37°C until clarified before use.
3. **Preparation of working solution 1 (for serum/plasma samples)::** Before testing, prepare sufficient working solution 1 according to the number of test wells. For example, to prepare 270 µL of working solution 1, thoroughly mix 90 µL of MTB reagent and 180 µL of alkali reagent. The working solution 1 should be prepared fresh for each assay.
4. **Preparation of working solution 2 (for tissue samples)::** Before testing, prepare sufficient working solution 2 according to the number of test wells. For example, to prepare 279 µL of working solution 2, thoroughly mix 90 µL of MTB reagent, 180 µL of alkali reagent and 9 µL of clarificant. The working solution 2 should be prepared fresh for each assay.
5. **Preparation of standard curve::** Always prepare a fresh set of standards. Discard working standard dilutions after use. Dilute 2.5 mmol/L standard solution with deionized water to serial concentrations. The recommended dilution gradient is as follows: 0, 0.2, 0.3, 0.4, 0.6, 0.8, 1, 1.2 mmol/L.

7. Sample preparation

Sample preparation:

Serum, plasma and urine: Detect directly. If not analyzed on the same day, serum or plasma can be stored at -80°C for one month.

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 deionized water with a dounce homogenizer at 4°C.

4. Centrifuge at 10,000 xg for 10 min to remove insoluble material. Collect supernatant and keep 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):

Sample type - Dilution factor

Dog serum - 2-3

Human serum - 3-6

Mouse serum - 3-6

Human urine - 4-8

20% Animal tissue homogenate - 1

Note: The diluent is deionized water. 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 calcium contamination during the experiment.
2. Severe hemolysis, jaundice and lipidemia will affect the experimental results.
3. Use deionized water as the homogenization medium to avoid calcium contamination when preparing tissue homogenates.

9. Operating steps

For serum (plasma) and other liquid samples:

① Standard wells: Add 10 μL of standard solution with different concentrations to the corresponding wells.

Sample wells: Add 10 μL of sample to the corresponding wells.

② Add 250 μL of working solution 1 into each well.

③ Mix thoroughly for 30 s with microplate reader and incubate for 5 min at room temperature.

④ Measure the OD value at 610 nm with microplate reader.

2. For tissue homogenate samples:

① Standard wells: Add 10 μL of standard solution with different concentrations to the corresponding wells.

Sample wells: Add 10 μL of sample to the corresponding wells.

② Add 250 µL of working solution 2 into each well.

③ Mix thoroughly for 30 s with microplate reader and incubate for 5 min at room temperature.

④ Measure the OD value at 610 nm with 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 and corresponding concentrations as y-axis and x-axis respectively. Create the standard curve (y = ax + b) with graph software (or EXCEL).

The sample:

1. Serum (plasma) sample and other liquid samples:

Calcium content (mmol/L) = (ΔA_610 - b) ÷ a × f

2. Tissue and cell samples:

Calcium content (mmol/g_{prot}) = (ΔA_610 - b) ÷ a × f ÷ C_{pr}

[Note]

f: Dilution factor of sample before test.

C_{pr}: Concentration of protein in sample, g_{prot}/L

ΔA_610: Absolute OD (OD_{Sample} – OD_{Blank}).

11. 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)	0.35	0.86	1.05
%CV	4.8	4.7	4.6

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)	0.35	0.86	1.05
%CV	8.4	8.6	8.5

Recovery

Three samples of high, middle and low concentrations were tested with 6 parallel replicates for each concentration to obtain an average recovery rate of 99%.

Recovery

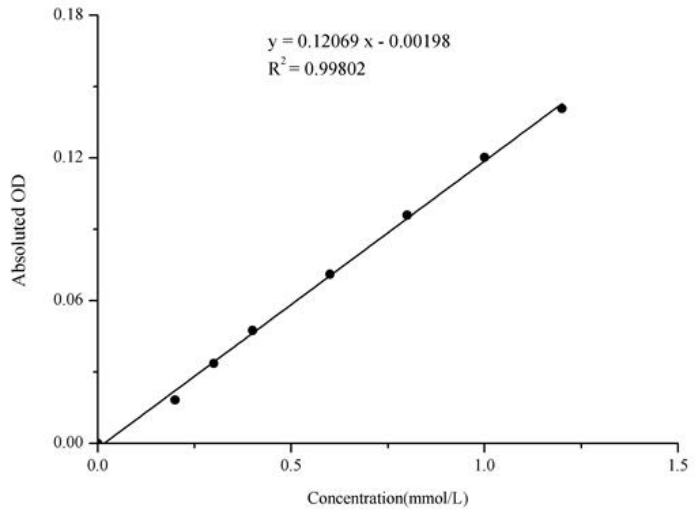
	Standard 1	Standard 2	Standard 3
Expected Conc. (mmol/L)	0.25	0.5	0.95
Observed Conc. (mmol/L)	0.3	0.5	0.9
Recovery rate (%)	101	99	97

Sensitivity

The analytical sensitivity of the assay is 0.07 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

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:



Standard curve data

Concentration (mmol/L)	OD value		Average OD	Absolute OD
0	0.225	0.227	0.226	0
0.2	0.246	0.242	0.244	0.018
0.3	0.265	0.255	0.260	0.034
0.4	0.273	0.275	0.274	0.047
0.6	0.297	0.297	0.297	0.071
0.8	0.321	0.323	0.322	0.096
1.0	0.348	0.344	0.346	0.120
1.2	0.365	0.369	0.367	0.141

12. Appendix II Example Analysis

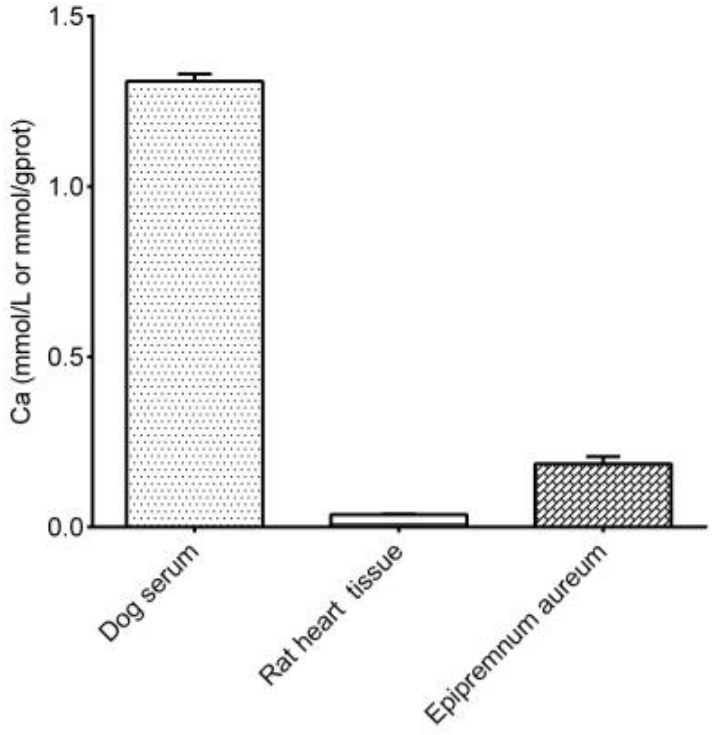
Example analysis:

Dilute dog serum with deionized water 2-fold, then add 10 µL of diluted sample and perform the assay according to the operating steps. The results are as follows:

Standard curve: y = 0.1298x + 0.0018, the average OD value of the sample well is 0.308, the average OD value of the blank well is 0.221, and the calculation result is:

Calcium content (mmol/L) = (0.308 - 0.221 - 0.0018) ÷ 0.1298 × 2 = 1.31 mmol/L

Detect dog serum (diluted 2-fold), 20% rat heart tissue homogenate (protein concentration is 9.30 g_{prot}/L), and 20% epipremnum aureum tissue homogenate (protein concentration is 4.34 g_{prot}/L) according to the protocol. The results are as follows:



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.