Technical Manual

Calcium (Ca) Colorimetric Assay Kit

- **Catalogue Code: MAES0091**
- **Size: 96T**
- **Research Use Only**

1. Key features and Sample Types

Detection method:

Colorimetric method

Specification:

96T

Range:

0.07-1.2 mmol/L

Sensitivity:

0.07 mmol/L

Storage:

2-8°C for 6 months

Expiry:

See Kit Label

Experiment Notes:

This kit is for **research use only.**

Instructions should be strictly followed. Changes of operation may result in unreliable results.

The validity of kit is 6 months.

Do not use components from different batches of kit.

2. Background

Calcium is an important multifunctional signaling molecule required for biological functions such as muscle contraction, nerve conduction and blood regulation. Calcium-mediated signal transduction is the basis of many basic processes of mammalian development and reproduction. Researches show that calcium signaling plays an important role in cancer. Excessive serum calcium may be the result of excessive intake of vitamin D and calcium, increased parathyroid hormone secretion, or bone destruction after tumor metastasis to bone. Inadequate intake of calcium or vitamin D may lead to hypocalcemia.

3. Intended Use

The kit is used for the determination of calcium content in serum, plasma, urine, cell culture supernatant, tissue and cells samples.

4. Detection Principle

Calcium ion in the sample bind to Methyl Thymol Blue (MTB) in alkaline solution and form blue complex. The blue complex has a specific absorption peak at 610 nm and calcium content can be calculated by measuring the OD value at 610 nm.

5. Kit components & storage

Item	Specification	Storage
MTB Reagent	10 mL × 1 vial	2-8°C, 6 months, avoid direct sunlight
Alkali Reagent	20 mL × 1 vial	2-8°C, 6 months
Clarificant	1 mL × 1 vial	2-8°C, 6 months
Calcium Standard (2.5 mmol/L)	10 mL × 1 vial	2-8°C, 6 months
Microplate	96 wells	No requirement
Plate Sealer	2 pieces	

Materials required but not supplied

- Micropipettor
- Incubator
- Centrifuge
- Microplate Reader (600-620 nm)
- Tips (10 µL, 200 µL, 1000 µL)
- EP tubes (1.5 mL, 2 mL)
- Double distilled water

6. Assay Notes:

Use deionized water as homogenized medium to prevent calcium contamination when preparing tissue/cell homogenates.

7. Reagent preparation:

1. Bring 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:** Mix MTB reagent and alkali reagent at a ratio of 1:2 fully. Prepare fresh solution before use. (For serum/plasma sample.)

4. **Preparation of working solution 2:** Mix MTB reagent, alkali reagent and clarificant at a ratio of 10:20:1 fully. Prepare fresh solution before use. (For tissue/cells sample.)
   .

8. Sample Preparation

1. Serum sample:
Fresh blood should be incubated at 25°C for 30 min to clot the blood. Centrifuge the sample at 2000 g for 15 min at 4°C. Take the serum (which is the upper light yellow clarified liquid layer) to preserve it on ice for detection. If not detected on the same day, the serum can be stored at -80°C for a month.

2. Plasma sample:
Take fresh blood into the tube which has anticoagulant (heparin is recommended), centrifuge at 700-1000 g for 10 min at 4°C. Take the plasma (which is the upper light yellow clarified liquid layer, don't take white blood cells and platelets in the middle layer) to preserve it on ice for detection. If not detected on the same day, the plasma can be stored at -80°C for a month.

3. Urine sample:
Collect fresh urine and centrifuge at 10000 g for 10 min at 4°C. Take the supernatant to preserve it on ice for detection. If not detected on the same day, the urine can be stored at -80°C for a month.

4. Cell culture supernatant:
Detect directly. If there is turbidity, centrifuge at 3100 g for 10 min. Take the supernatant to preserve it on ice for detection. If not detected on the same day, it can be stored at -80°C for a month.

5. Cell sample:
Collect the cells and wash the cells with PBS (0.01 M, pH 7.4) for 1~2 times. Centrifuge at 1000 g for 10 min and then discard the supernatant and keep the cell sediment. Add homogenization medium at a ratio of cell number (1×10^6): deionized water (μL) =1: 200. Sonicate the sample on an ice water bath. Centrifuge at 10000 g for 10 min, then take the supernatant and preserve it on ice for detection. If not detected on the same day, the cells sample (without homogenization) can be stored at -80°C for a month.

6. Tissue sample:
Take 0.02-1g fresh tissue to wash with PBS (0.01 M, pH 7.4) at 2-8°C. Absorb the water with filter paper and weigh. Homogenize at the ratio of the volume of deionized water (2-8°C) (mL): the weight of the tissue (g) =4:1, then centrifuge the tissue homogenate for 10 min at 10000 g at 4°C. Take the supernatant to preserve it on ice for detection. If not detected on the same day, the tissue sample (without homogenization) can be stored at -80°C for a month.

Sample Notes:

The concentration should be determined before preforming the assay. If the sample concentration is not within the range of the standard curve, users must determine the optimal sample dilutions for their particular experiments.

If the sample type is not included in the manual, a preliminary experiment is suggested to verify the validity.

Dilution of Samples:

Large variances in results may be seen when performing pre-experiments. Dilute the sample according to the result of the pre-experiment and the detection range (0.07-1.2 mmol/L).

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
HepG2 cell culture supernatant	2-4
20% Animal tissue homogenate	1
NRK cells homogenate (1.66 gprot/L)	1

Note: The diluent is deionized water.

9. Assay Protocol

Ambient Temperature: 25-30°C

Optimum detection wavelength: 610 nm

Plate Set Up:

	1	2	3	4	5	6	7	8	9	10	11	12
A	A	A	S1	S9	S17	S25	S33	S41	S49	S57	S65	S73
B	B	B	S2	S10	S18	S26	S34	S42	S50	S58	S66	S74
C	C	C	S3	S11	S19	S27	S35	S43	S51	S59	S67	S75
D	D	D	S4	S12	S20	S28	S36	S44	S52	S60	S68	S76
E	E	E	S5	S13	S21	S29	S37	S45	S53	S61	S69	S77
F	F	F	S6	S14	S22	S30	S38	S46	S54	S62	S70	S78
G	G	G	S7	S15	S23	S31	S39	S47	S55	S63	S71	S79
H	H	H	S8	S16	S24	S32	S40	S48	S56	S64	S72	S80

Note: A-H, standard wells; S1-S80, sample wells.

10. Operation Steps

The preparation of standard curve

Dilute calcium standard (2.5 mmol/L) with deionized water to a serial concentration. The recommended dilution gradient is as follows: 0, 0.2, 0.3, 0.4, 0.6, 0.8, 1, 1.2 mmol/L.

The measurement of samples

For serum (plasma) and other liquid sample

1. **Standard tube:** Take 10 µL of standard solution with different concentrations to the corresponding wells.
   Sample tube: Take 10 µL of sample to the corresponding wells.

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

3. Mix fully for 30 s with microplate reader and stand for 5 min at room temperature.

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

For tissue/cell homogenate sample

1. **Standard tube:** Take 10 µL of **standard solution** with different concentrations to the corresponding wells.
   Sample tube: Take 10 µL of **sample** to the corresponding wells.

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

3. Mix fully for 30 s with microplate reader and stand for 5 min at room temperature.

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

Operation Table

For serum (plasma) and other liquid sample

	Standard well	Sample well
Calcium standard solution with different concentrations (µL)	10	
Sample (µL)		10
Working solution 1 (µL)	250	250
Mix fully for 30 s with microplate reader and stand for 5 min. Measure the OD value of each well at 610 nm with microplate reader.		

For tissue/cell homogenate sample

	Standard well	Sample well
Calcium standard solution with different concentrations (µL)	10	
Sample (µL)		10
Working solution 2 (µL)	250	250
Mix fully for 30 s with microplate reader and stand for 5 min. Measure the OD value of each well at 610 nm with microplate reader.		

11. Calculations

Plot the standard curve by using OD value of standard and correspondent concentration as y-axis and x-axis respectively. Create the standard curve with graph software (or EXCEL). The concentration of the sample can be calculated according to the formula based on the OD value of sample. The standard curve is: y= ax + b.

1. Serum (plasma) and other liquid sample:

Calcium content (mmol/L)
$= (\Delta A_{610} - b) \div a \times f$

2. Tissue and cells sample:

Calcium content (mmol/gprot)
$= (\Delta A_{610} - b) \div a \times f \div C_{pr}$

y: $OD_{Standard} - OD_{Blank}$

x: The concentration of standard

a: The slope of standard curve

b: The intercept of standard curve

f: Dilution factor of sample before test

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

ΔA_{610}: Absolute OD ($OD_{Sample} - OD_{Blank}$)

12. Performance Characteristics

Detection Range	0.07-1.2 mmol/L
Sensitivity	0.07 mmol/L
Average recovery rate (%)	99
Average inter-assay CV (%)	8.5
Average intra-assay CV (%)	4.7

Analysis

Dilute dog serum with deionized water for 2 times, then take 10 µL of diluted sample, carry the assay according to the operation table.

The results are as follows:
Standard curve: y = 0.1298 x + 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

Safety Notes

Some of the reagents in the kit contain dangerous substances. Prevent touching skin and clothing.

Wash immediately with plenty of water if touching it carelessly.

All the samples and waste material should be treated according to the relevant rules of laboratory's biosafety.

Before the experiment, read the instructions carefully, and wear gloves and work clothes.

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