Calcium (Ca) Colorimetric Assay Kit

Catalog No: E-BC-K103-M

Method: Colorimetric method

Specification: 96T (Can detect 80 samples without duplication)

Measuring instrument: Microplate reader

Sensitivity: 0.07 mmol/L

Detection range: 0.07-1.2 mmol/L

▶ Please kindly provide us the lot number (on the outside of the box) of the kit for more efficient service.

General information

▲ Intended use

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

▲ 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.

▲ Detection principle

Calcium ion in the sample bind to methyl thymol blue (MTB) in alkaline solution and form blue complex. Calcium content can be calculated by measuring the OD value at 610 nm.

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▲ Kit components & storage

Item	Component	Specification	Storage
Reagent 1	MTB Reagent	10 mL × 1 vial	2-8 , 6 months, shading light
Reagent 2	Alkali Reagent	20 mL × 1 vial	2-8 , 6 months
Reagent 3	Clarificant	1 mL × 1 vial	2-8 , 6 months
Reagent 4	2.5 mmol/L Calcium Standard	10 mL × 1 vial	2-8 , 6 months
	Microplate	96 wells	No requirement
	Plate Sealer	2 pieces	

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.

▲ Materials prepared by users

Instruments

Microplate reader (600-620 nm), Micropipettor, Centrifuge, Incubator, Vortex mixer

Consumptive material

Tips (10 μL, 200 μL, 1000 μL), EP tubes (1.5 mL, 2 mL, 5 mL)

Reagents

Deionized water

▲ **The key points of the assay**

1. Avoid calcium contamination in the experiment. Disposable 96 wells microplate is recommended.

2. Severe hemolysis, jaundice and lipidemia will affect the experiment result.

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

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Pre-assay preparation

▲ Reagent preparation

1. The reagent 3 will be solid at 2-8   , preheat the reagent 3 at 37   until clarified before use.

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

3. Preparation of working solution 2

   Mix reagent 1, reagent 2 and reagent 3 at a ratio of 10:20:1 fully. Prepare fresh solution before use. (For tissue/cells sample.)

▲ Sample preparation

The samples should be prepared as conventional methods. Also please refer to appendix II.

Sample requirements

1. It is recommended to use heparin as an anticoagulant, do not use calcium chelating agent and oxalate.

2. Samples can be stored at 2~8   for 3~4 days or at - 2 0   for several months.

▲ Dilution of sample

It is recommended to take 2~3 samples with expected large difference to do pre-experiment before formal experiment and 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.

Assay protocol	
Ambient temperature	25-30
Optimum detection wavelength	610 nm

Instructions for the use of transferpettor

(1) Equilibrate the pipette tip in that reagent before pipetting each reagent.

(2) Don't add the liquid outside the tips into the reaction system when pipetting each reagent.

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Assay protocol

▲**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.

▲Operating steps

The preparation of standard curve

Dilute 2.5 mmol/L calcium standard 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

1.For serum(plasma) and other liquid sample

(1) Standard well: Take 10 μL of standard solution with different concentrations to the corresponding wells.

   Sample well: 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.

2. For tissue/cell homogenate sample

(1) Standard well: Take 10 μL of standard solution with different concentrations to the corresponding wells.

   Sample well: 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**

1. 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.		

2. 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.		

▲ Calculation

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

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

Note:

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 ($OD_{Sample} - OD_{Blank}$).

▲ Notes

1. This kit is for research use only.

2. Instructions should be followed strictly, changes of operation may result in unreliable results.

3. The validity of kit is 6 months.

4. Do not use components from different batches of kit.

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Appendix I Performance characteristics

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

▲ **Example analysis**

Dilute dog serum with deionized water for 2 times, then take 10 µL of diluted sample and 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:

$$\text{Calcium content (mmol/L)} = \frac{0.308 - 0.221 - 0.0018}{0.1298} \times 2 = 1.31 \text{ mmol/L}$$

Appendix II Sample preparation

The following sample pretreatment methods are for reference only.

▲ Serum

Collect fresh blood and stand at 25 for 30 min to clot the blood. Then centrifuge at 2000 g for 15 min at 4 . 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 for a month.

▲ Plasma

Take fresh blood into the tube which has anticoagulant (heparin is recommended), centrifuge at 700-1000 g for 10 min at 4 . 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 for a month.

▲ Urine

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

▲ Cell culture supernatant

Collect cell culture supernatant and centrifuge at 10000 g for 10 min at 4 . Take the supernatant to preserve it on ice for detection. If not detected on the same day, the urine can be stored at -80 for a month.

▲ Tissue sample

Take 0.02-1g fresh tissue to wash with deionized water at 2-8   . Absorb the water with filter paper and weigh. Homogenize at the ratio of the volume of deionized water (2-8   ) (mL): the weight of the tissue (g) =4:1, then centrifuge the tissue homogenate for 10 min at 10000 g at 4   . Take the supernatant to preserve it on ice for detection. Meanwhile, determine the protein concentration of supernatant (E-BC-K318-M, E-BC-K168-M, E-BC-K165-M). If not detected on the same day, the tissue sample (without homogenization) can be stored at -80   for a month.

▲ Cells

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 deionized water at a ratio of cell number (10^6): deionized water (μL) =1: 300-500. Sonicate or grind with hand-operated in ice water bath. Centrifuge at 10000 g for 10 min, then take the supernatant and preserve it on ice for detection. Meanwhile, determine the protein concentration of supernatant (E-BC-K318-M, E-BC-K168-M, E-BC-K165-M). If not detected on the same day, the cells sample (without homogenization) can be stored at -80   for a month.

Note:

Homogenized method:

1. Hand-operated: Weigh the tissue and mince to small pieces (1 mm^3), then put the tissues pieces to glass homogenized tube. Add homogenized medium into homogenized tube, place the tube into the ice bath with left hand, and insert the glass tamping rod vertically into the homogenized tube with the right hand to grind up and down for 6-8 min.

   Or put the tissue into the mortar, and add liquid nitrogen to grind fully. Then add the homogenized medium to homogenize.

2. Mechanical homogenate: Weigh the tissue to EP tube, add the homogenized medium to homogenize the tissue with homogenizer instrument (60 Hz, 90s) in the ice bath. (For samples of skin, muscle and plant tissue, the time of homogenization can be properly prolonged.)

3. Ultrasonication: Treat the cells with ultrasonic cell disruptor (200 W, 2 s/ time, interval for 3 s, the total time is 5 min).

▲ **Note for sample**

1. Please predict the concentration before assaying. If the sample concentration is not within the range of the standard curve, users must determine the optimal sample dilutions for their particular experiments.

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

3. If a lysis buffer is used to prepare tissue or cell homogenates, there is a possibility of causing a deviation due to the introduced chemical substance.