（FOR RESEARCH USE ONLY. DO NOT USE IT IN CLINICAL DIAGNOSIS !）

Catalog No：E-BC-K103-M

Specification：48T(32 samples)/96T(80 samples)/ 500Assays(484 samples)

Measuring instrument：Microplate reader（600-620 nm）

Detection range：0.07-1.2 mmol/L

Elabscience® Calcium (Ca) Colorimetric Assay Kit

This manual must be read attentively and completely before using this product.
If you have any problem, please contact our Technical Service Center for help：

Toll-free: 1-888-852-8623
Tell: 1-832-243-6086
Fax: 1-832-243-6017
Email: techsupport@elabscience.com
Website: www.elabscience.com

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

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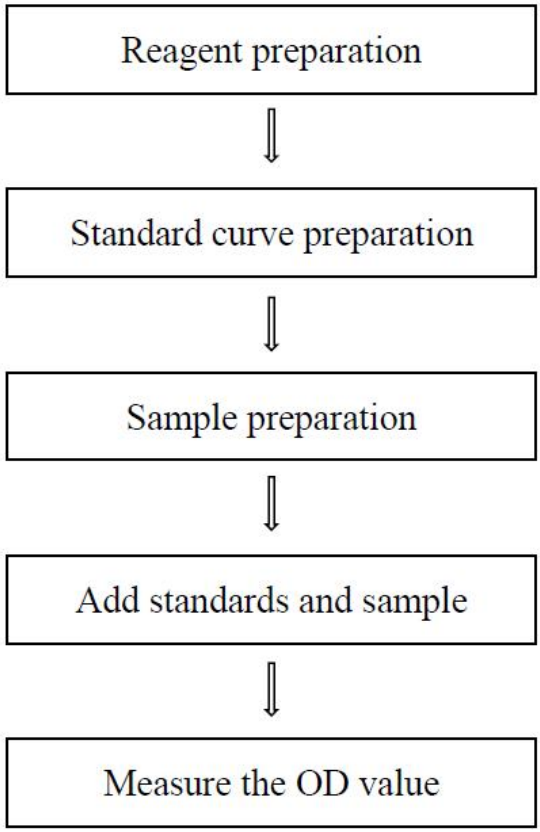
Assay summary



Intended use

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

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.

Kit components & storage

Item	Component	Size 1(48 T)	Size 2(96 T)	Size 3 (500Assays)	Storage
Reagent 1	MTB Reagent	5 mL×1 vial	10 mL×1 vial	10 mL×5 vials	2-8°C, 12 months, shading light
Reagent 2	Alkali Reagent	10 mL×1 vial	20 mL×1 vial	50 mL×2 vials	2-8°C, 12 months
Reagent 3	Clarificant	1 mL×1 vial	1 mL×1 vial	5 mL×1 vial	2-8°C, 12 months
Reagent 4	2.5 mmol/L Calcium Standard	5 mL×1 vial	10 mL×1 vial	50 mL×1 vial	2-8°C, 12 months
	Microplate	48 wells	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. For a small volume of reagents, please centrifuge before use, so as not to obtain sufficient amount of reagents.

Materials prepared by users

Instruments:

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

Reagents:

Deionized water

Reagent preparation

① Equilibrate all the 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 The preparation of working solution 1：

Before testing, please prepare sufficient working solution 1 according to the test wells. For example, prepare 270 μL of working solution 1 (mix well 90 μL of MTB reagent and 180 μL of alkali reagent). The working solution 1 should be prepared on spot. (For serum/plasma sample)

④ The preparation of working solution 2：

Before testing, please prepare sufficient working solution 2 according to the test wells. For example, prepare 279 μL of working solution 2 (mix well 90 μL of MTB reagent, 180 μL of alkali reagent and 9 μL of clarificant). The working solution 2 should be prepared on spot. (For tissue sample)

⑤ The 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 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. Reference is as follows:

Item	①	②	③	④	⑤	⑥	⑦	⑧
Concentration (mmol/L)	**0**	**0.2**	**0.3**	**0.4**	**0.6**	**0.8**	**1.0**	**1.2**
2.5 mmol/L standard (μL)	0	40	60	80	120	160	200	240
Deionized water (μL)	500	460	440	420	380	340	300	260

Sample preparation

① Sample preparation

Serum, plasma and urine: detect directly. If not detected on the same day, the serum or plasma can be stored at -80°C for a 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 10000×g for 10 min to remove insoluble material. Collect supernatant and keep it on ice for detection.

⑤ Meanwhile, determine the protein concentration of supernatant (E-BC-K318-M).

② 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 do pretest to confirm the dilution factor.

The key points of the assay

① Avoid calcium contamination in the experiment.

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 homogenates.

Operating steps

1. 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.

2. For tissue 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.

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 absoluted OD value.

3. Plot the standard curve by using absoluted OD value of standard and correspondent concentration 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:

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

2. Tissue and cell samples:

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

[Note]

f: Dilution factor of sample before test.

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

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

Appendix I Performance Characteristics

1. Parameter:

Intra-assay Precision

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

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.

Parameters	Sample 1	Sample 2	Sample 3
Mean (mmol/L)	0.35	0.86	1.05
%CV	8.4	8.6	8.5

Recovery

Take three samples of high concentration, middle concentration and low concentration to test the samples of each concentration for 6 times parallelly to get the average recovery rate of 99%.

	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

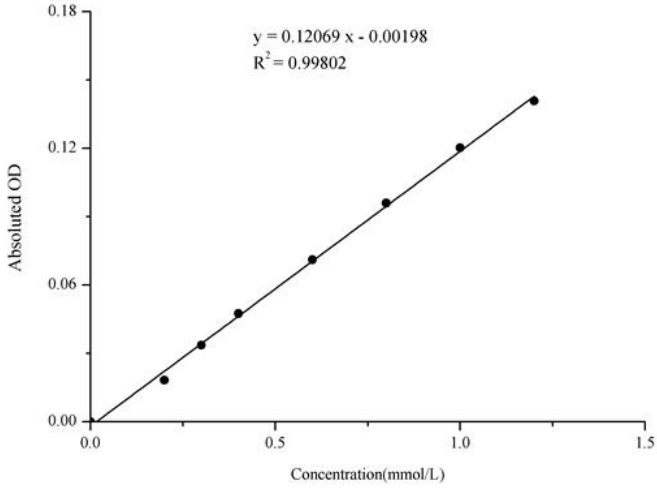
Sensitivity

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.

2. Standard curve:

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), so the standard curve and data are provided as below for reference only:

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



Appendix II Example Analysis

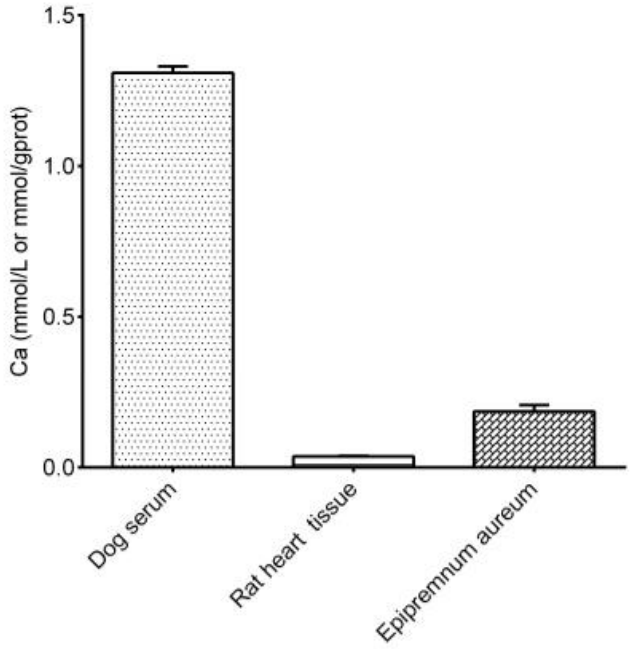
Example analysis：

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

Detect dog serum (dilute for 2 times), 20% rat heart tissue homogenate (the concentration of protein is 9.30 gprot/L), and 20% epipremnum aureum tissue homogenate (the concentration of protein is 4.34 gprot/L) according to the protocol, the result is as follows：



Appendix III Publications

1. Mao J , Sun Z , Wang S ,et al.Multifunctional Bionic Periosteum with Ion Sustained‐Release for Bone Regeneration[J].Adv. Sci. 11.DOI:10.1002/advs.202403976.
2. Lin B , Li F , Hui J ,et al.Modular Reconfigurable Approach Toward Noninvasive Wearable Body Net for Monitoring Sweat and Physiological Signals[J].
3. Kang J Y , Gu J Y , Baek D C ,et al.A Capsicum annuum L. seed extract exerts anti-neuroexcitotoxicity in HT22 hippocampal neurons[J].Food & Function, 2024, 15(4):10.DOI:10.1039/D3FO04501C.

Statement

1. This assay kit is for Research Use Only. We will not response for any arising problems or legal responsibilities causing by using the kit for clinical diagnosis or other purpose.
2. Please read the instructions carefully and adjust the instruments before the experiments. Please follow the instructions strictly during the experiments.
3. Protection methods must be taken by wearing lab coat and latex gloves.
4. If the concentration of substance is not within the detection range exactly, an extra dilution or concentration should be taken for the sample.
5. It is recommended to take a pre-test if your sample is not listed in the instruction book.
6. The experimental results are closely related to the situation of reagents, operations, environment and so on. Elabscience will guarantee the quality of the kits only, and NOT be responsible for the sample consumption caused by using the assay kits. It is better to calculate the possible usage of sample and reserve sufficient samples before use.