CheKine™ Mirco Calcium Content Assay Kit

Cat #: KTB1117 Size: 48 T/48 S 96 T/96 S

	Mirco Calcium Content Assay Kit		
REF	**Cat #:** KTB1117	LOT	**Lot #:** Refer to product label
	Applicable sample: Animal and Plant Tissues, Cells, Plasma, Serum or other Liquid samples		
	Storage: Stored at 4℃ for 6 months, protected from light		

Assay Principle

Calcium (calcium) is typically the most abundant mineral in the bodies of mammals and is involved in regulating numerous cellular life processes. It is also one of the most important intracellular regulatory factors. Calcium can exist in two forms: as free ions or as complexes with bound calcium ions, such as calcium phosphate and calcium carbonate, which are components of bone tissue. A wide range of physiological processes, including muscle contraction, cell adhesion, hormone/neurotransmitter release, glycogen metabolism, cell proliferation/differentiation, blood coagulation, neural or synaptic transmission, and bone structure support, are regulated by calcium signaling. Defects in the integrity of cell-specific calcium signaling systems can lead to the development of various diseases. Although 99% of the calcium in the human body is found in bones and teeth, clinical attention is often focused on blood calcium levels, known as serum calcium. Blood calcium levels are primarily regulated by parathyroid hormone, which controls the storage or release of calcium from bones to prevent hypercalcemia or hypocalcemia. Calcium in serum exists primarily in three forms: approximately 50% as free calcium ions, about 45% as protein-bound calcium, and about 5% as calcium complexes (mainly calcium citrate). Calcium levels are typically inversely related to phosphorus levels. CheKine™ Mirco Calcium Content Assay Kit provides a simple, convenient, and rapid method for detecting calcium ion content, suitable for animal and plant tissues, cells, serum (plasma) or other Liquid samples. The principle is based on the reaction of calcium ions with o-cresolphthalein complexone under alkaline conditions to form a purple complex. The calcium ion content can be quantitatively determined by measuring the absorbance at 575 nm.

Materials Supplied and Storage Conditions

Kit components	Size		Storage conditions
	48 T	96 T	
Reagent Ⅰ	18 mL	36 mL	4℃, protected from light
Reagent Ⅱ	Powder×1 vial	Powder×1 vial	4℃, protected from light
Reagent Ⅲ	Powder×1 vial	Powder×1 vial	4℃, protected from light
Standard	1.5 mL	1.5 mL	4℃

Note: Before formal testing, it is recommended to select 2-3 samples with large expected differences for pre-experiment.

Materials Required but Not Supplied

- Microplate reader or visible spectrophotometer capable of measuring absorbance at 575 nm
- 96-well plate or microglass cuvette, precision pipettes, disposable pipette tips
- Analytical balance, low-temperature centrifuge, ice maker
- Deionized water
- Homogenizer (for tissue samples)

Reagent Preparation

Reagent I: Ready to use as supplied. Equilibrate to room temperature before use. Store at 4℃, protected from light.

Working Reagent II: Prepared before use. Add 15 mL of Reagent I to 48 T and 30 mL of Reagent I to 96 T, dissolve completely, prepare freshly and use immediately.

Working Reagent III: Prepared before use. Add 14 mL of Working Reagent II to 48 T and 28 mL of Working Reagent II to 96 T, Dissolve completely and keep for use. Store protected from light at 4℃ for up to 1 week.

Standard: 20 μmol/mL calcium standard solution. Ready to use as supplied. Equilibrate to room temperature before use. Store at 4℃.

Standard Preparation: 20 μmol/mL calcium standard solution. Dilute further according to the table below to prepare the standards:

Num.	Standard (μL)	Deionized water (μL)	Concentration (μmol/mL)
Std.1	50 μL 20 μmol/mL Standard	450	2
Std.2	200 μL of Std.1 (2 μmol/mL)	200	1
Std.3	200 μL of Std.2 (1 μmol/mL)	200	0.5
Std.4	200 μL of Std.3 (0.5 μmol/mL)	200	0.25
Std.5	200 μL of Std.4 (0.25 μmol/mL)	200	0.125
Std.6	200 μL of Std.5 (0.125 μmol/mL)	200	0.063
Std.7	200 μL of Std.6 (0.063 μmol/mL)	200	0.031
Std.8	0	200	0 (Blank Well)

Sample Preparation

Note: Use fresh samples whenever possible. If not assayed immediately, samples can be stored at -80℃ for 1 month. When measuring, the temperature and time of thawing should be controlled. When thawing at room temperature, the sample should be thawed within 4 h.

1. Animal and Plant Tissues: Weigh approximately 0.1 g of the sample, add 1 mL of deionized water, homogenize on ice, centrifuge at 12,000 g for 10 min at 4℃, and collect the supernatant. Keep the supernatant on ice for testing.
2. Cells: Collect 10 million cells in a centrifuge tube, wash the cells with cold PBS, centrifuge, and discard the supernatant. Add 1 mL of deionized water, sonicate the cells on ice for 5 min (power 20% or 200 W, pulse 3 s, pause 7 s, repeat 30 times), then centrifuge at 12,000 g for 10 min at 4℃, and collect the supernatant. Keep the supernatant on ice for testing.
3. Serum (Plasma) and Other Liquid Samples: Test directly.

Note:1. When preparing serum, it is recommended to use heparin and avoid chelating agents like EDTA, as chelating agents can bind to calcium ions and affect the test results.

2. If the protein concentration of the sample is need to determined, it is recommended to use Abbkine Cat #: KTD3001 Protein Quantification Kit (BCA Assay) to measure the protein concentration of the sample.

Assay Procedure

1. Preheat the microplate reader or visible spectrophotometer for more than 30 min, and adjust the wavelength to 575 nm. Visible spectrophotometer was returned to zero with deionized water.

2. Sample measurement. (The following operations are operated in a 96-well plate or microglass cuvettes):

Reagent	Test Well (μL)	Standard Well (μL)
Sample	4	0
Standard	0	4
Working Reagent III	196	196
Mix thoroughly and let stand for 5 min. Measure the absorbance at 575 nm for each well using a 96-well plate or a microglass cuvette. Record the absorbance values as A_{Test}, $A_{Standard}$ and A_{Blank}. Calculate $\Delta A_{Test}=A_{Test}-A_{Blank}$, $\Delta A_{Standard}=A_{Standard}-A_{Blank}$.		

Note: Before the experiment, it is recommended to perform a preliminary test with 2-3 samples that are expected to show significant differences. If ΔA_{Test} is less than 0.005, increase the sample amount appropriately. If ΔA_{Test} is greater than 0.6, dilute the sample further with deionized water, and multiply the calculated result by the dilution factor, or reduce the amount of sample used for extraction.

Data Analysis

Note: We provide you with calculation formulae, including the derivation process and final formula. The two are exactly equal. It is suggested that the concise calculation formula in bold is final formula.

1. Drawing of standard curve:

With the concentration of the standard solution as the x-axis and the $\Delta A_{Standard}$ as the y-axis, draw the standard curve, get the standard equation, and bring the ΔA_{Test} into the equation to get the x value (μmol/mL).

(1) Calculated by fresh weight of samples

Calcium ion (μmol/g fresh weight)**=x÷W×F**

(2) Calculated by protein concentration

Calcium ion (μmol/mg prot)**=x÷Cpr×F**

(3) Calculated by volume of liquid samples

Calcium ion (μmol/mL)**=x×F**

(4) Calculated by number of cells

Calcium ion (μmol/10^4)**=x÷N×F**

Where: W: weight of sample, g; F: the sample dilution factor; Cpr: sample protein concentration, mg/mL; N: total number of cells, 10^4.

Typical Data

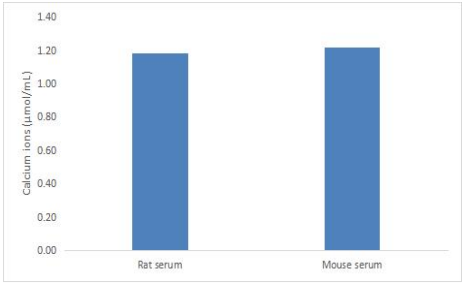

Figure 1. Determination calcium ion content in Rat serum and Mouse serum by this assay kit

Recommended Products

Catalog No.	Product Name
KTB1015	CheKine™ Micro α-Glucosidase Activity Assay Kit
KTB1121	CheKine™ Pyruvate Acid (PA) Colorimetric Assay Kit

Disclaimer

The reagent is only used in the field of scientific research, not suitable for clinical diagnosis or other purposes. For your safety and health, please wear a lab coat and disposable gloves.