(Catalog # : CA01ME/CA02ME)
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Instruction manual

* FOR RESEARCH USE ONLY
* STORE AT 4℃ UPON ARRIVAL

Calcium (Ca) Assay Kit LS (CPZⅢ)

(Chlorophosphonazo-III Chromogenic method)

Description

Calcium is the most abundant and one of the most important minerals in the human body. Approximately 99% of body calcium is found in bones. The calcium level in the extracellular space is in dynamic equilibrium with the rapidly exchangeable fraction of bone calcium. Calcium ions affect the contractility of heart and skeletal musculature and are essential for the function of the nervous system. Calcium ion play an important role in blood clotting and bone minimization. In plasma, calcium is bound to a considerable extent to proteins, 10 % is in the form of inorganic complexes and 50 % is present as free ion species. The calcium homeostasis regulated by the parathyroid hormone (PTH), calcitriol(CT), and calcitonin. A decrease in albumin level causes a decrease in serum calcium. Low levels of calcium are found in hypoparathyroidism, pseudohypoparathyroidism, vitamin D deficiency, malnutrition and intestinal malabsorption. Among causes of hypercalcemia are cancers, large intake of vitamin D, enhanced renal retention, osteoporosis, sarcosidosis, thyrotoxicosis, hyperparathyroidism.

This product is a direct colorimetric assay kit without deproteinization of the sample. Calcium with Chlorophosphonazo-III (as chelator) at neutral pH, yields a blue colored complex.The intensity of the color formed is proportional to the calcium concentration in the sample.

Kit contents

250 tests （Catalog # : CA02ME ）

R-R Chelate color (Chlorophosphonazo-III) ●	60 mL×1
STD Calcium Standard 10 mg/dL ●	1.0 mL×1

(Catalog # : CA01ME)=(Catalog # : CA02ME) ×2

Note

A) Unstableness of incubation temperature may result in unstable results.
B) Use disposable test tube and glassware washed with 1M HNO_3 or 1M HCl solution and distilled water.
C) Accuracy in pipetting volume for samples and reagents may affect the quality of assay. Please note that samples, standards and Working Reagent must be poured accurately µL level.
D) Temperature for chromogen reaction may affect optical density. Please try to extend or shorten chromogen reaction time depending on room temperature.
E) In the cell lysate or the tissue extract use as specimen, high concentration of proteins or lipid, may affect observed value. Please remove its by ultrafiltration or centrifugation.

Operation

1. Sample preparation

◇Serum or Plasma

Insoluble substances in serum and plasma samples should be removed by filtration or centrifugation. EDTA-plasma cannot be used.

◇Tissue extract, Lysate, Other samples.

Urine (24 hour pooled urine), or other biological fluid:

Add 6M HCl to the sample and adjust pH 2.0-3.0 (e.g. 5-10µL 6M HCl/ 1mL of lysate.). Centrifuge at 6,000 rpm for 15 min. Collect the supernatant and use it for assay.

Tissue:

Add 5% TCA solution, vortex 1 min. and incubate at 4-8°C for 30 min. Centrifuge at 6,000 rpm for 15 min. Collect the supernatant and use it for assay.

* Sample pH should be between pH2 to pH8.

2. Assay preparation

Bring all reagents to room temperature before use.

3. Assay procedure.

Procedure using microplate reader.

(1 assay sample 242µL)

○Assay

(1) Add 2 µL of Distilled water (Blank) / STD (Standard)/ sample into each well.

(2) Add 240 µL of R-R to each well and incubate at room temperature for 10 min.

(3) Read the absorbance at 690 nm (main) and 750 nm (sub). --> OD

* Select the filter: 680-700 nm at 690nm (main), 740-800 nm at 750 nm (sub).

		Assay Sample		
Add (µL)		Blank OD_{Bl}	Standard OD_{Std}	Sample OD_S
1	Distilled water	2	-	-
	STD	-	2	-
	Assay sample	-	-	2
2	R-R	240	240	240
↓				
Mix and incubate for 10 minutes at room temperature. Read the absorbance at 690 nm (main) and 750 nm (sub). (Possible ranges of wavelength for select the filter : 680-700 nm at 690nm, 740-800 nm at 750 nm.)				

○Calculations

$\Delta OD_{Std} = OD_{Std} - OD_{Bl}$

$\Delta OD_S = OD_S - OD_{Bl}$

Calcium (mg/dL) = $\Delta OD_S/\Delta OD_{Std}$ X 10

Calcium (mM) = $\Delta OD_S/\Delta OD_{Std}$ X 2.495

(Assay example)

	OD (690nm)	OD (750nm)	OD	ΔOD	Calcium (mg/dL)
Blank	0.846	0.074	0.776	-	-
Standard	1.060	0.074	0.986	0.210	-
Sample	1.000	0.073	0.927	0.151	7.19

***Observed 690 nm with 750 nm**

[OD = OD(690nm) - OD(750nm)]

ΔOD_{Std} = (1.060 – 0.074) – (0.846 - 0.074) =0.210

ΔOD_S = (0.609 – 0.067) – (0.480 - 0.064) =0.151

$Calcium_{Sample}$ (mg/dL) = $\Delta OD_S/\Delta OD_{Std}$ x 10

= 0.151/ 0.210 x 10 = 7.19 (mg/dL)

$Calcium_{Sample}$ (mM) = $\Delta OD_S/\Delta OD_{Std}$ x 2.495

= 0.151 / 0.210 x 2.495 = 1.79 (mM)

***Observed 690 nm only**

[OD = OD(690nm)]

ΔOD_{Std} = 1.060 – 0.846 = 0.214

ΔOD_S = 1.000 – 0.846 = 0.154

$Calcium_{Sample}$ (mg/dL) = $\Delta OD_S/\Delta OD_{Std}$ x 10

= 0.154 / 0.214 x 10 = 7.20 (mg/dL)

$Calcium_{Sample}$ (mM) = $\Delta OD_S/\Delta OD_{Std}$ x 2.495

= 0.154 / 0.214 x 2.495 = 1.80 (mM)

*In diluted sample of seminal fluid, multiply the result by dilution-factor.

Performance

Measuring range 0.2 – 30 mg/dL

Imprecision Imprecision was evaluated using commercially available quality control serum.

Within run

	Mean mg/dL	S.D	C.V %
Level 1	6.3	0.26	4.09
Level 2	12.6	0.4	3.32

Interferences No interference by the note of substances were observed.
Conjugated bilirubin and unconjugated bilirubin 40 mg/dL
Hemoglobin 1 g/dL Chyle 1,000 FTU

Expiration date and preservation conditions

Storage conditions: Store at 2-8°C. Don't freeze.

Expiration: 1 year from the date of manufacture.
After the bottles are opened,
the kit should be used in 1 month.

Reference

1.) J. W. Ferguson, J. J. Richard, J. W. O'laughlin and C. V. Banks: Simultaneous Spectrophotometric Determination of Calcium and Magnesium with Chlorophosphonazo-III, Anal. Chem, 36, 796.2 (1962).

2.) D. S. Howell, J. C. Pita, J. F. Marquez, "Ultramicro Spectrophotometric Determination of Calcium in Biologic Fluids", Anal. Chem, 38, 434 (1966).

3.) Fujita. T, Noguchi. K, Terashima. I : Apoplastic mesophyll signals induce rapid stomatal responses to CO2 in Commelina communis, *New Phytol,* 199(2), p395-406 (2013).

4.) K. Hisano, O. Fujise, M. Miura, T. Hamachi, E. Matsuzaki andF. Nishimura: *Molecular Oral Microbiology,* 29(2), p79-89 (2014).

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