MC Reagent Biochemical Assay Series

Calcium Assay Kit

Chlorophosponazo-III Chromogenic method

Biochemical Significance and Test Summary

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 bone calcium. Calcium ions affect the contractility of heart and skeletal muscles and are essential for the function of the nervous system. Calcium ions 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 malabsortion. 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.

1. Kit contents (200 tests)

R-1	Chelate color	1 x 50 mL	Ready to use
STD	10 mg/dL Ca Standard	1 x 1.0 mL	Ready to use

*Storage conditions: Store at 2-8°C. **Don't freeze**.
*Expiration: 1 year. After the vials are opened, the kit should be used in one month.
*Measuring range: 0.05-20 mg/dL

2. Materials required but not provided

(1) Distilled water
(2) Micropipettors and pipette tips
(3) Clear flat-bottom 96-well plate
(4) Microplate reader with 690 nm capability

3. Assay preparation

Bring all reagents to room temperature before use.

4. Sample preparation

Serum/ Plasma: Insoluble substances in serum and plasma samples should be removed by filtration or centrifugation. EDTA-plasma cannot be used.
Urine (24 hour pooled urine) Dilute the sample 1/2 in distilled water. Add 6M HCl to the diluted sample and adjust pH 2.0-3.0 (e.g. 5-10 µL of 6M HCl/1mL of lysate). Centrifuge at 6,000 rpm for 15 minutes. Collect the supernatant and use it for assay.
Biological fluid: Add 6M HCl to the sample and adjust pH 2.0-3.0 (e.g. 5-10 µL of 6M HCl/1mL of lysate). Centrifuge at 6,000 rpm for 15 minutes. Collect the supernatant and use it for assay.
Tissue: Add 3% TCA solution, vortex for 1 minute and incubate for 30 minutes at 4-8°C. Centrifuge at 6,000 rpm for 15 minutes. Collect the supernatant and use it for assay.

Note: Sample pH should be between pH 2 and pH 8.

5. Assay protocol

(1) Add 5 µL of Distilled water(Blank)/STD(Standard)/Sample into each well.
(2) Add 250 µL of R-1 to each well and incubate for 10 minutes at room temperature.

(3) Read the absorbance at 690 nm (680-700 nm) and 750 nm (740-800 nm, reference wavelength) . ----- OD

6. Calculations

$\Delta OD_{Standard} = OD_{Standard} - OD_{Blank}$, $\Delta OD_{Sample} = OD_{Sample} - OD_{Blank}$

Calcium (mg/dL) = $\Delta OD_{Sample} / \Delta OD_{Standard}$ x 10
Calcium (mM) = $\Delta OD_{Sample} / \Delta OD_{Standard}$ x 2.495

(Assay example)

	OD (690 nm)	OD (750 nm)	OD	Δ OD	Calcium (mg/dL)
DW (Blank)	0.505	0.066	0.439	-	-
Standard	0.991	0.078	0.913	0.474	-
Sample	0.837	0.073	0.764	0.325	6.86

(a) Measurement at 690 nm and 750 nm (reference wavelength):

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

$\Delta OD_{Standard}$ = (0.991 - 0.078) - (0.505 - 0.066) = 0.913
ΔOD_{Sample} = (0.837 - 0.073) - (0.505 - 0.066) = 0.764

$Calcium_{Sample} = \Delta OD_{Sample}/\Delta OD_{Standard}$ x 10 = (0.325/ 0.474) x 10 = 6.86 (mg/dL)
$Calcium_{Sample}$ (mM) = $\Delta OD_{Sample}/\Delta OD_{Standard}$ x 2.495 = (0.325/ 0.474) x 2.495 = 1.71 (mM)

(b) Measurement at 690 nm:

$\Delta OD_{Standard}$ = 0.991 - 0.505 = 0.486
ΔOD_{Sample} = 0.837 - 0.505 = 0.332

$Calcium_{Sample} = \Delta OD_{Sample}/\Delta OD_{Standard}$ x 10 = (0.332/ 0.486) x 10 = 6.83 (mg/dL)
$Calcium_{Sample}$ (μM) = $\Delta OD_{Sample}/\Delta OD_{Standard}$ x 2.495 = (0.332/ 0.486) x 2.495 = 1.70 (mM)

7. Interferences

EDTA inhibits calcium to chromogenic system. The test is not affected by presence of bilirubin-F and bilirubin-C up to 40 mg/dL, hemoglobin up to 1 g/dL and chyle up to 1,000 FTU.

8. Quality Control

Use of control sera is recommended to monitor the quality of assay results.

9. 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 CO_2 in Commelina communis, *New Phytol*, 199(2), p395-406 (2012).

10. Technical support & troubleshooting

(1) Unstableness of incubation temperature may result in unstable results.
(2) Use disposable test tube and glassware washed with 1M HNO_3 or 1M HCl, and rinse with distilled water.
(3) Accuracy to the microliter is important to obtain good results. Ensure maximum precision when pipetting.
(4) Temperature for the chromogenic reaction may affect the optical density. It may be necessary to adjust the reaction time depending on the room temperature.
(5) High concentration of proteins or lipid in cell lysate or in tissue extract may affect the observed value. Please remove them by ultrafiltration or centrifugation.
(6) 24 hour pooled urine: Dilute the sample 1/2 in distilled water. Mix. Multiply results by 2 (dilution factor) for assay sample.

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