



PRODUCT INFORMATION

Calcium Assay (Chlorophosphonazo-III Chromogenic Method)

Cat. No. KT-763 / KT-764

INTENDED USE

The Calcium Assay (Chlorophosphonazo-III Chromogenic Method) is a direct colorimetric assay for the quantitative determination of calcium in biological samples that does not require sample deproteinization. For research use only. Not for use in diagnostic procedures in the U.S.

PRINCIPLE

Calcium forms a blue chelate with the Chlorophosphonazo-III chromogen at neutral pH. The intensity of this colored complex is proportional to the calcium concentration in the sample and is measured at 690 nm.

COMPONENTS

	250 tests	500 tests
	<u>KT-763</u>	<u>KT-764</u>
1. Chromogen (Chlorophosphonazo-III), 60 mL	x1	x2
2. Calcium Calibrator (10 mg/dL), 1.0 mL	x1	x2

Store all kit components at 4°C.

PRECAUTIONS

1. Fluctuating incubation temperature may result in variable results.
2. Use disposable test tubes and glassware washed with 1M HNO₃ or 1M HCl solution and distilled water.
3. Sample and reagent pipetting accuracy may affect assay performance. Please note that samples, calibrator, and reagents must be dispensed accurately at the μ L level.

4. The temperature of the reaction may affect the O.D. reading. Please extend or shorten the chromogen reaction time depending on the ambient room temperature if necessary.
5. For cell lysates or the tissue extraction samples, a high concentration of proteins or lipids may affect the assay result. For best results, remove proteins or lipids by ultrafiltration or centrifugation.

SAMPLE PREPARATION

1. Serum or plasma

Insoluble substances in serum and plasma samples should be removed by filtration or centrifugation. EDTA-plasma samples cannot be used as EDTA interferes with this assay.

2. Tissue extracts, cell lysates, and other samples such as urine or other biological fluids:

If the sample is turbid, centrifuge at 6,000 rpm for 15 min. Collect the supernatant and use for the assay

If necessary, add small amounts of 6M HCl to the sample and adjust pH to 2.0 - 3.0. For example, add ~5-10 μ L of 6M HCl per 1 mL of sample.

3. Tissue samples

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 for the assay.

Sample pH should be between pH 2.0 to pH 8.0.

REAGENT PREPARATION

Reagents are ready to use.

Bring all reagents to room temperature before use.

ASSAY PROTOCOL (Microplate and Microplate Reader)

(Total reaction volume = 242 µL)

1. Add 2 µL of Blank (purified water), Calcium Calibrator, or Sample to each well.
2. Add 240 µL of Chromogen to each well, mix, and incubate at room temperature for 10 minutes. Mix carefully using a pipette to avoid foaming. If a plate mixer is used for mixing, there is a risk of obtaining poor reproducibility.
3. Read the OD absorbance at 690 nm (main) and 750 nm (sub). Acceptable wavelength range: 680 - 700 nm (main) and 740 - 800 nm (sub).

Assay Protocol				
Step	(µL)	Blank	Calibrator	Sample
1	Purified water	2	-	-
	Calcium Calibrator	-	2	-
	Sample	-	-	2
	Chromogen	240	240	240
2	Mix and incubate for 10 minutes at room temp.			
3	Read the OD absorbance at 690 nm (main) and 750 nm (sub).			

CALCULATION OF SAMPLE CONCENTRATION

$$\frac{(\text{OD}_{690} \text{ sample} - \text{OD}_{750} \text{ sample}) - (\text{OD}_{690} \text{ blank} - \text{OD}_{750} \text{ blank})}{(\text{OD}_{690} \text{ calib.} - \text{OD}_{750} \text{ calib.}) - (\text{OD}_{690} \text{ blank} - \text{OD}_{750} \text{ blank})} \times 10 = \text{Calcium (mg/dL)}$$

Unit Conversion:

$$\text{Calcium (mg/dL)} \times 0.2495 = \text{Calcium (mM)}$$

Assay Example

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

When assaying diluted samples, multiply the result by the dilution factor.

PERFORMANCE

Assay Range: 0.2 - 30 mg/dL

Precision: Precision was evaluated using commercially available quality control serum.

Within Run Precision	Mean (mg/dL)	S.D.	C.V.%
Level 1	6.3	0.26	4.09
Level 2	12.6	0.4	3.32

Interference:

Conjugated bilirubin	No interference up to at least 40 mg/dL
Unconjugated bilirubin	No interference up to at least 40 mg/dL
Hemoglobin	No interference up to at least 1 g/dL
Chyle	No interference up to at least 1,000 FTU

Shelf life:

Until expiration date at 4°C. After opening any of the kit components, store at 4°C and use within one month. **Do not freeze.****FOR RESEARCH USE ONLY.****Not for use in diagnostic procedures in the U.S.****KAMIYA BIOMEDICAL COMPANY**

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