



PRODUCT INFORMATION

Calcium Assay (O-Cresolphthalein Chromogenic Method)

Cat. No. KT-765 / KT-766

INTENDED USE

The Calcium Assay (O-Cresolphthalein 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 violet chelate with the O-Cresolphthalein chromogen at alkaline pH. The intensity of this colored complex is proportional to the calcium concentration in the sample and is measured at 570 nm.

COMPONENTS

	250 tests	500 tests
	<u>KT-765</u>	<u>KT-766</u>
1. Buffer, 48 mL	x1	x2
2. Chromogen (O-Cresolphthalein), 13 mL	x1	x2
3. Calcium Calibrator (10 mg/dL), 1.4 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 3% 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 = 245 µL)

1. Add 5 µL of Blank (purified water), Calcium Calibrator, or Sample to each well.
2. Add 190 µL of Buffer to each well, mix, and incubate at room temperature for 5 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. Add 50 µL of Chromogen to each well, mix, and incubate at room temperature for 5 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.
4. Read the OD absorbance at 570 nm (main) and 700 nm (sub). Acceptable wavelength range: 560 - 590 nm (main) and 650 - 700 nm (sub).

Assay Protocol				
Step	(µL)	Blank	Calibrator	Sample
1	Purified water	5	-	-
	Calcium Calibrator	-	5	-
	Sample	-	-	5
2	Buffer	190	190	190
	Mix and incubate for 5 minutes at room temp.			
3	Chromogen	50	50	50
	Mix and incubate for 5 minutes at room temp.			
4	Read the OD absorbance at 570 nm (main) and 700 nm (sub).			

CALCULATION OF SAMPLE CONCENTRATION

$$\frac{(\text{OD}_{570} \text{ sample} - \text{OD}_{700} \text{ sample}) - (\text{OD}_{570} \text{ blank} - \text{OD}_{700} \text{ blank})}{(\text{OD}_{570} \text{ calib.} - \text{OD}_{700} \text{ calib.}) - (\text{OD}_{570} \text{ blank} - \text{OD}_{700} \text{ blank})} \times 10 = \text{Calcium (mg/dL)}$$

Unit Conversion:

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

Assay Example

	OD (570 nm)	OD (700 nm)	OD	ΔOD	Calcium (mg/dL)
Blank	0.655	0.034	0.621	-	-
Calibrator	1.135	0.036	1.099	0.478	-
Sample	0.973	0.036	0.937	0.316	6.61

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	6.5	0.3	4.6
Level 2	11.9	0.4	3.1

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 0.2 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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