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Product Information

Calcium Colorimetric Assay Kit

Catalog Number **MAK022**
Storage Temperature 2–8 °C

TECHNICAL BULLETIN

Product Description

Calcium, the most abundant mineral in the human body, is a crucial intracellular element that is responsible for regulating many cellular processes. Calcium is found in either the free ion form or in bound complexes, for example the calcium phosphate and calcium carbonate complexes that make up bone tissue. Numerous physiological processes, including muscle contraction, cell adhesion, hormones/ neurotransmitters release, glycogen metabolism, cell proliferation/differentiation, blood clotting, nerve or synapthetic impulse transmission, and structural support of the skeleton are regulated by calcium signaling. Defects in the integrity of cell-specific calcium signaling systems may be associated with certain human diseases.

In this assay, the calcium ion concentration is determined by the chromogenic complex formed between calcium ions and *o*-cresolphthalein, which is measured at 575 nm and is proportional to the concentration of calcium ions present. The linear range of detection for this kit is between 0.4–2.0 µg.

This kit is suitable for use with cell and tissue culture supernatants, urine, plasma, serum, fecal material, media, and other biological fluids.

Components

The kit is sufficient for 250 assays in 96 well plates.

Calcium Assay Buffer Catalog Number MAK022A	15 mL
Chromogenic Reagent Catalog Number MAK022B	25 mL
Calcium Standard, 500 mM Catalog Number MAK022C	0.1 mL

Reagents and Equipment Required but Not Provided.

- 96 well flat-bottom plate – It is recommended to use clear plates for colorimetric assays.
- Spectrophotometric multiwell plate reader

Precautions and Disclaimer

This product is for R&D use only, not for drug, household, or other uses. Please consult the Material Safety Data Sheet for information regarding hazards and safe handling practices.

Preparation Instructions

Briefly centrifuge vials before opening.

Calcium Assay Buffer – Allow buffer to come to room temperature before use.

Chromogenic Reagent – Allow to come to room temperature before use.

Storage/Stability

The kit is shipped on wet ice. Storage at 2-8 °C, protected from light, is recommended.

Procedure

All samples and standards should be run in duplicate. Use ultrapure water for the preparation of all reagents.

Calcium Standards for Colorimetric Detection

Dilute 10 µL of the 500 mM Calcium Standard Solution with 990 µL of water to prepare a 5 mM (0.2 µg/µL) Calcium Standard Solution. Mix well by pipetting. Add 0, 2, 4, 6, 8, and 10 µL of the 5 mM standard solution into a 96 well plate, generating 0 (assay blank), 0.4, 0.8, 1.2, 1.6, and 2.0 µg/well standards. Bring the volume to a total of 50 µL with water.

Sample Preparation

Serum or urine samples can be used directly in this assay. For other liquid samples, add 2–50 µL samples to well. Bring samples to a final volume of 50 µL with water.

Samples can be assayed without any prior treatment.

Note: Some MRI contrast agents can cause transient interference in the assay.

Assay Reaction

1. Add 90 µL of the Chromogenic Reagent to each well containing standards, samples, or controls. Mix gently.

2. Add 60 µL of Calcium Assay Buffer to each well and mix gently.

3. Incubate the reaction for 5–10 minutes at room temperature. Protect the plate from light during incubation.

4. Measure the absorbance at 575 nm (A_{575}).

Note: Read standard and samples within 30 minutes as the chromophore is unstable and will fade over time.

Results

Calculations

The background for the assays is the value obtained for the 0 (assay blank) Calcium Standard. Correct for the background by subtracting the 0 (assay blank) value from all readings. Background values can be significant and must be subtracted from all readings. Use the values obtained from the appropriate Calcium standards to plot a standard curve.

Note: A new standard curve must be set up each time the assay is run.

Concentration of Calcium

$$S_a/S_v = C$$

S_a = Amount of calcium in unknown sample (µg) from standard curve
S_v = Sample volume (µL) added into the wells
C = Concentration of calcium in sample

Calcium molecular weight: 40 µg/µmole

Sample Calculation

Amount of Calcium (S_a) = 0.580 µg (from standard curve)
Sample volume (S_v) = 50 µL

Concentration of Calcium in sample

$$0.580\ \mu g/50\ \mu L = 0.0116\ \mu g/\mu L$$

$$0.0116\ \mu g/\mu L/40\ \mu g/\mu mole = 0.00029\ \mu mole/\mu L = 0.29\ nmole/\mu L$$

Troubleshooting Guide

Problem	Possible Cause	Suggested Solution
Assay not working	Ice Cold Assay Buffer	Assay Buffer must be at room temperature
	Omission of step in procedure	Refer and follow Technical Bulletin precisely
	Plate reader at incorrect wavelength	Check filter settings of instrument
	Type of 96 well plate used	For colorimetric assays, use clear plates
Samples with erratic readings	Samples prepared in different buffer	Use the Assay Buffer provided or refer to Technical Bulletin for instructions
	Cell/Tissue culture samples were incompletely homogenized	Repeat the sample homogenization, increasing the length and extent of homogenization step.
	Samples used after multiple freeze-thaw cycles	Aliquot and freeze samples if samples will be used multiple times
	Presence of interfering substance in the sample	If possible, dilute sample further
	Use of old or inappropriately stored samples	Use fresh samples and store correctly until use
Lower/higher readings in samples and standards	Improperly thawed components	Thaw all components completely and mix gently before use
	Use of expired kit or improperly stored reagents	Check the expiration date and store the components appropriately
	Allowing the reagents to sit for extended times on ice	Prepare fresh Master Reaction Mix before each use
	Incorrect incubation times or temperatures	Refer to Technical Bulletin and verify correct incubation times and temperatures
	Incorrect volumes used	Use calibrated pipettes and aliquot correctly
Non-linear standard curve	Use of partially thawed components	Thaw and resuspend all components before preparing the reaction mix
	Pipetting errors in preparation of standards	Avoid pipetting small volumes
	Pipetting errors in the Reaction Mix	Prepare a Master Reaction Mix whenever possible
	Air bubbles formed in well	Pipette gently against the wall of the tubes
	Standard stock is at incorrect concentration	Refer to the standard dilution instructions in the Technical Bulletin
	Calculation errors	Recheck calculations after referring to Technical Bulletin
	Substituting reagents from older kits/lots	Use fresh components from the same kit
Unanticipated results	Samples measured at incorrect wavelength	Check the equipment and filter settings
	Samples contain interfering substances	If possible, dilute sample further
	Sample readings above/below the linear range	Concentrate or dilute samples so readings are in the linear range

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