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 synaptic 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 (A575).

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
\[ \frac{S_a}{S_v} = C \]

\( S_a \) = 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
\[ \frac{0.580 \, \mu g}{50 \, \mu L} = 0.0116 \, \mu g/\mu L \]

\[ 0.0116 \, \mu g/\mu L \times 40 \, \mu g/\mu mole = 0.00029 \, \mu mole/\mu L \]

\[ = 0.29 \, n mole/\mu L \]
<table>
<thead>
<tr>
<th>Problem</th>
<th>Possible Cause</th>
<th>Suggested Solution</th>
</tr>
</thead>
<tbody>
<tr>
<td>Assay not working</td>
<td>Ice Cold Assay Buffer</td>
<td>Assay Buffer must be at room temperature</td>
</tr>
<tr>
<td></td>
<td>Omission of step in procedure</td>
<td>Refer and follow Technical Bulletin precisely</td>
</tr>
<tr>
<td></td>
<td>Plate reader at incorrect wavelength</td>
<td>Check filter settings of instrument</td>
</tr>
<tr>
<td></td>
<td>Type of 96 well plate used</td>
<td>For colorimetric assays, use clear plates</td>
</tr>
<tr>
<td>Samples with erratic readings</td>
<td>Samples prepared in different buffer</td>
<td>Use the Assay Buffer provided or refer to Technical Bulletin for instructions</td>
</tr>
<tr>
<td></td>
<td>Cell/Tissue culture samples were incompletely homogenized</td>
<td>Repeat the sample homogenization, increasing the length and extent of homogenization step.</td>
</tr>
<tr>
<td></td>
<td>Samples used after multiple freeze-thaw cycles</td>
<td>Aliquot and freeze samples if samples will be used multiple times</td>
</tr>
<tr>
<td></td>
<td>Presence of interfering substance in the sample</td>
<td>If possible, dilute sample further</td>
</tr>
<tr>
<td></td>
<td>Use of old or inappropriately stored samples</td>
<td>Use fresh samples and store correctly until use</td>
</tr>
<tr>
<td>Lower/higher readings in samples and standards</td>
<td>Improperly thawed components</td>
<td>Thaw all components completely and mix gently before use</td>
</tr>
<tr>
<td></td>
<td>Use of expired kit or improperly stored reagents</td>
<td>Check the expiration date and store the components appropriately</td>
</tr>
<tr>
<td></td>
<td>Allowing the reagents to sit for extended times on ice</td>
<td>Prepare fresh Master Reaction Mix before each use</td>
</tr>
<tr>
<td></td>
<td>Incorrect incubation times or temperatures</td>
<td>Refer to Technical Bulletin and verify correct incubation times and temperatures</td>
</tr>
<tr>
<td></td>
<td>Incorrect volumes used</td>
<td>Use calibrated pipettes and aliquot correctly</td>
</tr>
<tr>
<td>Non-linear standard curve</td>
<td>Use of partially thawed components</td>
<td>Thaw and resuspend all components before preparing the reaction mix</td>
</tr>
<tr>
<td></td>
<td>Pipetting errors in preparation of standards</td>
<td>Avoid pipetting small volumes</td>
</tr>
<tr>
<td></td>
<td>Pipetting errors in the Reaction Mix</td>
<td>Prepare a Master Reaction Mix whenever possible</td>
</tr>
<tr>
<td></td>
<td>Air bubbles formed in well</td>
<td>Pipette gently against the wall of the tubes</td>
</tr>
<tr>
<td></td>
<td>Standard stock is at incorrect concentration</td>
<td>Refer to the standard dilution instructions in the Technical Bulletin</td>
</tr>
<tr>
<td></td>
<td>Calculation errors</td>
<td>Recheck calculations after referring to Technical Bulletin</td>
</tr>
<tr>
<td></td>
<td>Substituting reagents from older kits/lots</td>
<td>Use fresh components from the same kit</td>
</tr>
<tr>
<td>Unanticipated results</td>
<td>Samples measured at incorrect wavelength</td>
<td>Check the equipment and filter settings</td>
</tr>
<tr>
<td></td>
<td>Samples contain interfering substances</td>
<td>If possible, dilute sample further</td>
</tr>
<tr>
<td></td>
<td>Sample readings above/below the linear range</td>
<td>Concentrate or dilute samples so readings are in the linear range</td>
</tr>
</tbody>
</table>