Calcium Assay Kit

Catalog Number KA1644

500 assays

Version: 05

Intended for research use only
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Introduction

Intended Use

Application:
✓ Direct Assays: Ca^{2+} in blood, urine, saliva etc.
✓ Drug Discovery/Pharmacology: effects of drugs on calcium metabolism.
✓ Food and Beverages: calcium determination.
✓ Environment: calcium determination in water and soil.

Features:
✓ Sensitive and accurate: Use as little as 5 μL samples. Linear detection range 0.08 mg/dL (20 μM) to 20 mg/dL (5 mM) Ca^{2+} in 96-well plate assay.
✓ Simple and high-throughput: The procedure involves addition of a single working reagent and incubation for 3 min. Can be readily automated as a high-throughput assay for thousands of samples per day.
✓ Improved reagent stability and versatility: The optimized formulation has greatly enhanced reagent and signal stability. Cuvet or 96-well plate assay.
✓ Low interference in biological samples. No pretreatments are needed. Assays can be directly performed on raw biological samples i.e., in the presence of lipid, protein and minerals such as magnesium, iron and zinc.

Background

CALCIUM is measured to monitor diseases of the bone or calcium regulation disorders. Increased calcium levels in serum are reported in hyperparathyroidism, metastatic bone lesions and hypervitaminosis, while decreased levels are observed in hypoparathyroidism, nephrosis, rickets, steatorrhea, nephritis and calcium-losing syndromes. Urinary calcium levels aid the clinician in understanding how the kidneys handle calcium in certain diseases of the parathyroid gland. Urinary calcium levels are also essential in the medical evaluation of kidney stones.

Simple, direct and automation-ready procedures for measuring calcium concentration in biological samples are becoming popular in Research and Drug Discovery.

Principle of the Assay

The Calcium Assay Kit is designed to measure calcium directly in biological samples without any pretreatment. A phenolsulphonephthalein dye in the kit forms a very stable blue colored complex specifically with free calcium. The intensity of the color, measured at 612 nm, is directly proportional to the calcium concentration in the sample. The optimized formulation minimizes any interference by substances such as magnesium, lipid, protein and bilirubin.
General Information

Materials Supplied

List of component

<table>
<thead>
<tr>
<th>Component</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reagent A</td>
<td>50 mL</td>
</tr>
<tr>
<td>Reagent B</td>
<td>50 mL</td>
</tr>
<tr>
<td>Calcium standard: 20 mg/dL Ca$^{2+}$</td>
<td>1 mL</td>
</tr>
</tbody>
</table>

Storage Instruction

Store Reagent and Standard at 4°C. Shelf life: 12 months after receipt.

Materials Required but Not Supplied

✓ Pipetting devices and accessories (e.g. 5 μL).
✓ Procedure using 96-well plate:
  Clear-bottom 96-well plates (e.g. Corning Costar) and plate reader.
✓ Procedure using cuvette:
  Cuvets and Spectrophotometer for measuring OD$_{612}$ nm.

Precautions for Use

✓ Reagents are for research use only. Normal precautions for laboratory reagents should be exercised while using the reagents. Please refer to Material Safety Data Sheet for detailed information.
✓ EDTA and other Ca$^{2+}$ chelators interfere with this assay. This assay can not be applied to plasma samples obtained with EDTA.
Assay Protocol

Assay Procedure

Matrix in certain samples (e.g. whole blood) may interfere with the assay. For internal standard protocols, please see "Product FAQ" for this product.

✓ Procedure using 96-well plate:
1. Dilute standards as follows. Transfer 5 μL diluted standards and samples into wells of a clear bottom 96-well plate. Store diluted standards at 4°C for future use.

<table>
<thead>
<tr>
<th>No</th>
<th>STD + H₂O</th>
<th>Vol (μL)</th>
<th>Ca (mg/dL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>100μL + 0μL</td>
<td>100</td>
<td>20</td>
</tr>
<tr>
<td>2</td>
<td>80μL + 20μL</td>
<td>100</td>
<td>16</td>
</tr>
<tr>
<td>3</td>
<td>60μL + 40μL</td>
<td>100</td>
<td>12</td>
</tr>
<tr>
<td>4</td>
<td>40μL + 60μL</td>
<td>100</td>
<td>8</td>
</tr>
<tr>
<td>5</td>
<td>30μL + 70μL</td>
<td>100</td>
<td>6</td>
</tr>
<tr>
<td>6</td>
<td>20μL + 80μL</td>
<td>100</td>
<td>4</td>
</tr>
<tr>
<td>7</td>
<td>10μL + 90μL</td>
<td>100</td>
<td>2</td>
</tr>
<tr>
<td>8</td>
<td>0μL + 100μL</td>
<td>100</td>
<td>0</td>
</tr>
</tbody>
</table>

2. Prepare enough working reagent by combining equal volumes of Reagent A and B. Add 200 μL working reagent and tap lightly to mix.
3. Incubate 3 min at room temperature and read optical density at 570-650nm (peak absorbance at 612nm).

✓ Procedure using cuvette:
1. Set up test tubes for diluted standards and Samples. Transfer 15 μL diluted Standards and samples to appropriately labeled tubes.
2. Add 1000 μL working reagent and vortex to mix. Incubate 3 min. Transfer to cuvet and read optical density at 612nm.
Data Analysis

Calculation of Results

Subtract blank OD (water, #8) from the standard OD values and plot the OD against Ca\(^{2+}\) standard concentrations. Determine the slope using linear regression fitting. Calcium concentration of the sample is calculated as

\[
\text{Slope} = \frac{\text{OD}_{\text{SAMPLE}} - \text{OD}_{\text{BLANK}}}{\text{OD}_{\text{SAMPLE}} - \text{OD}_{\text{BLANK}}} (\text{mg/dL})
\]

\(\text{OD}_{\text{SAMPLE}}\) and \(\text{OD}_{\text{BLANK}}\) are OD\(_{612nm}\) values of sample and sample blank (water or buffer in which the sample was diluted).

Conversions: 1 mg/dL Ca\(^{2+}\) equals 250 μM, 0.001% or 10 ppm.

• EXAMPLES

Samples were assayed in duplicate using the 96-well plate protocol. The Ca\(^{2+}\) values (mg/dL) were 8.5 ± 0.4 (rat serum), 6.5 ± 0.3 (human serum), 7.6 ± 0.1 (goat serum), 11.1 ± 1.0 (Invitrogen fetal bovine serum), 2.5 ± 0.4 (fresh human urine), 41.3 ± 0.5 (Kirkland 2% reduced fat milk), 5.0 ± 0.0 (tap water, Hayward, CA), 0.86 ± 0.07 (tap water, San Bruno, CA), 1.8 ± 0.1 (Crystal Geyser natural alpine spring water), 2.3 ± 0.1 (Coca-cola® classic coke), 0.04 ± 0.01 (Lipton Lemon iced tea), 0.52 ± 0.07 (soil extract. 5.6 g of Hayward, CA soil was extracted with 10 mL MilliQ water. The supernatant was centrifuged to remove any insoluble particles. Clear supernatant was assayed).

Standard Curve in 96-well plate assay
We have whole blood samples. Does your assay work?

Yes, the Calcium Assay Kit can be used on whole blood samples. To correct for interference in the sample matrix, two internal standard methods have been validated. Protocol A is quicker whereas Protocol B is slightly more involved, but requires less sample and is, thus, recommended for customer’s that have a limited quantity of sample. Additionally, protocol B requires less Reagent because each sample requires one well rather than three separate wells per sample. Please note that 20 mM EDTA is needed for this experiment and is not provided. The customer should prepare this solution.

- Protocol A: 3 Separate wells needed for each sample
  1. Whole Blood samples require an internal standard and need three separate reactions: 1) Sample plus Standard 2) Sample alone and 3) Sample Blank. For the internal standard prepare 250 µL 10 mg/dL Ca\(^{2+}\) Standard by mixing 125 µL 20 mg/dL Standard and 125 µL dH\(_2\)O. Transfer 5 µL whole blood sample to three separate wells. Add 5 µL of 10 mg/dL Ca\(^{2+}\) to the 1) Sample plus Standard well, 5 µL dH\(_2\)O to 2) Sample alone well and 5 µL 20 mM EDTA to 3) sample Blank well.
  2. Add 200 µL Working Reagent and tap lightly to mix. Note: If any particulates or turbidity are seen pipette up and down to dissolve.
  3. Incubate 3 min at room temperature and read optical density at 570-650 nm (peak absorbance at 612 nm).

- Protocol B: 1 Well needed for each sample
  1. Dilute standard to 10 mg/dL Ca\(^{2+}\) by mixing 125 µL 20 mg/dL Standard and 125 µL dH\(_2\)O.
  2. Transfer 5 µL whole blood sample to a well.
  3. Add 200 µL Working Reagent and tap lightly to mix. Note: If any particulates are seen pipette up and down to dissolve.
  4. Incubate 3 min at room temperature and read optical density at 570-650 nm (peak absorbance at 612 nm). OD\(_{\text{SAMPLE}}\)
  5. Carefully transfer 5 µL of 10 mg/dL standard to the sample well from step 2. Tap plate to mix. Repeat Step 4. OD\(_{\text{STANDARD}}\)
  6. Add 5 µL of 20 mM EDTA to the same well from step 2. Tap plate to mix. Repeat step 4. OD\(_{\text{BLANK}}\)

- Calculation
  The whole blood sample concentration is computed as follows:
  \[ [\text{Ca}^{2+}] = \frac{(\text{OD}_{\text{SAMPLE}} - \text{OD}_{\text{BLANK}})/(\text{OD}_{\text{STANDARD}} - \text{OD}_{\text{SAMPLE}}) \times 10 \times n (\text{mg/dL})} \]
Sample plus Standard respectively, 10 is the concentration of the standard in mg/dL, and n is the sample dilution factor. If the calculated calcium concentration is greater than 10 mg/dL, dilute sample in dH$_2$O and repeat assay. Multiply result by the dilution factor n.

- **Example**
  One human blood sample was assayed using the two methods. The Ca$^{2+}$ concentration was 8.48 mg/dL using Protocol A and 8.38 mg/dL using Protocol B.

- Is the calcium assay kit compatible with acids?
  Yes, our kit is compatible with acids, such as 0.5 M HCl.

- Do you know if phosphate in the sample will interfere with the calcium assay or if calcium in the sample will interfere with the phosphate assay?
  Phosphate (at least up to 30 mM) in the sample does not interfere with the calcium assay.

- Does freezing of serum have any impact on serum Ca compared to using fresh serum?
  Our assay kit measures the total calcium content of samples. In serum about half of the calcium is free ("ionized") and the other half is bound to proteins, especially albumin (~40%) or anions (~10%). Repeated freeze-thaw cycles may cause precipitation of proteins which would alter the outcome of the assay. We recommend avoiding repeated freeze thaw cycles for serum samples.