Calcium Assay Kit (Colorimetric)

Calcium Assay Kit (Colorimetric) is a detection kit for the quantification of Calcium in biological, food and environmental samples.

Catalog number: ARG82142

Package: 500 tests

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INTRODUCTION

Calcium is a chemical element with the symbol Ca and atomic number 20. As an alkaline earth metal, calcium is a reactive metal that forms a dark oxide-nitride layer when exposed to air. Its physical and chemical properties are most similar to its heavier homologues strontium and barium. It is the fifth most abundant element in Earth's crust, and the third most abundant metal, after iron and aluminium. The most common calcium compound on Earth is calcium carbonate, found in limestone and the fossilised remnants of early sea life; gypsum, anhydrite, fluorite, and apatite are also sources of calcium. The name derives from Latin calx "lime", which was obtained from heating limestone.

Calcium is the most abundant metal and the fifth-most abundant element in the human body. As electrolytes, calcium ions play a vital role in the physiological and biochemical processes of organisms and cells: in signal transduction pathways where they act as a second messenger; in neurotransmitter release from neurons; in contraction of all muscle cell types; as cofactors in many enzymes; and in fertilization. Calcium ions outside cells are important for maintaining the potential difference across excitable cell membranes, protein synthesis, and bone formation. [Provide by Wikipedia: Calcium]

PRINCIPLE OF THE ASSAY

This Calcium Assay Kit (Colorimetric) is a simple colorimetric assay that measures the amount of calcium present in biological, food and environmental samples. samples. 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 O.D. 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.

MATERIALS PROVIDED & STORAGE INFORMATION

The kit is shipped with blue ice. Store all components at 4°C upon receiving. Shelf life: 12 months after receipt.

Component	Quantity	Storage information
Reagent A	50 mL	4°C
Reagent B	50 mL	4°C
Standard (20 mg/dL Ca^{2+})	1 mL	4°C

MATERIALS REQUIRED BUT NOT PROVIDED

- Microplate reader capable of reading at O.D. 570-650 nm
- Centrifuge
- Clear flat-bottom 96 well microplate
- Deionized or Distilled water
- Pipettes, pipette tips and Multichannel micropipette reservoir
- 20 mM EDTA (Optional)

TECHNICAL NOTES AND PRECAUTIONS

- Wear protective gloves, clothing, eye, and face protection especially while handling blood or body fluid samples.
- EDTA and other Ca^{2+} chelators interfere with this assay. This assay can't be applied to plasma samples obtained with EDTA.
- 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.
- All reagents should be mixed by gentle inversion or swirling prior to use. Do not induce foaming.
- Before using the kit, spin tubes and bring down all components to the bottom of tubes.
- It is highly recommended assaying the Standards and samples in duplicates.
- Change pipette tips between the addition of different reagent or samples.

SAMPLE COLLECTION & STORAGE INFORMATION

The sample collection and storage conditions listed below are intended as general guidelines. Sample stability has not been evaluated.

Serum: Collect blood in a tube with no anticoagulant. Allow the blood to clot at room temperature for 30 minutes. Centrifuge at 2500 x g for 20 minutes at 4°C. Collect the serum and assay directly.

Plasma: Collect blood with heparin or citrate and centrifuge at 2000 x g for 10 minutes at 4°C. Collect the plasma layer and assay directly.

Whole Blood: whole blood contain matrix and need an internal standard for calculation, please refer ASSAY PROCEDURE for detail.

Note:

1. Matrix in certain samples (e.g. whole blood) may interfere with the assay. For internal standard protocol, please refer ASSAY PROCEDURE.
2. EDTA and other Ca^{2+} chelators interfere with this assay. This assay can't be applied to plasma samples obtained with EDTA.

REAGENT PREPARATION

- **Working Reagent:** prepare fresh Working Reagent for each reaction by mixing equal volumes of Reagent A and Reagent B. And 200 µL of mixed working Reagent is required for each well.
- **Standard:** Dilute standards as follows. Store diluted standards at 4°C for future use.

Standard tube	Ca^{2+} (mg/dL)	Distilled water (µL)	20 mg/dL Standard (µL)
S1	20	0	100
S2	16	20	80
S3	12	40	60
S4	8	60	40
S5	6	70	30
S6	4	80	20
S7	2	90	10
S8	0	100	0

ASSAY PROCEDURE

Equilibrate reagents to room temperature. Briefly centrifuge tubes before use.

For Samples without matrix:

1. Add **5 µL** of **Standards** and **samples** into wells of clear bottom 96-well microplate.
2. Add **200 µL** of **Working Reagent** to each well. Tap lightly to mix.
3. Incubate for **3 minutes** at **room temperature** and read the absorbance at **O.D. 612 nm** (570-650 nm)

For Samples with matrix (ex: Whole Blood) procedure

1. Dilute standard to **10 mg/dL** of Ca^{2+} by mixing **125 µL** of **20 mg/dL Standard** and **125 µL** of **distilled water.**
2. Transfer **5 µL** of **whole blood samples** to wells of clear bottom 96-well microplate.
3. Add **200 µL** of **Working Reagent** and tap lightly to mix.
   Note: If any particulates are seen pipette up and down to dissolve.
4. Incubate for **3 minutes** at **room temperature** and read the absorbance at **O.D. 612 nm** (570-650 nm). The results are OD_{SAMPLE}
5. Carefully transfer **5 µL** of **10 mg/dL standard** to the sample wells from step 2. Tap plate to mix.
6. Incubate for **3 minutes** at **room temperature** and read the absorbance at **O.D. 612 nm** (570-650 nm). The results are $OD_{STANDARD}$
7. Add **5 µL** of **20 mM EDTA** to the same wells from step 2. Tap plate to mix.
8. Incubate for **3 minutes** at **room temperature** and read the absorbance at **O.D. 612 nm** (570-650 nm). The results are OD_{BLANK}

CALCULATION OF RESULTS

1. Subtract blank OD (distilled water, S8) 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 follow:

   Ca^{2+} (mg/dL) = [(OD_{SAMPLE} – OD_{BLANK}) / Slope]

   Note:

   - OD_{SAMPLE}, OD_{BLANK}: the O.D. 612 nm values of the sample and sample blank (water (S8) or buffer in which the sample was diluted).

2. The whole blood sample (matrix containing samples) concentration is computed as follows:

   Ca^{2+} (mg/dL) = [(OD_{SAMPLE} – OD_{BLANK}) / ($OD_{STANDARD}$ – OD_{SAMPLE})] x 10 x n

   Note:

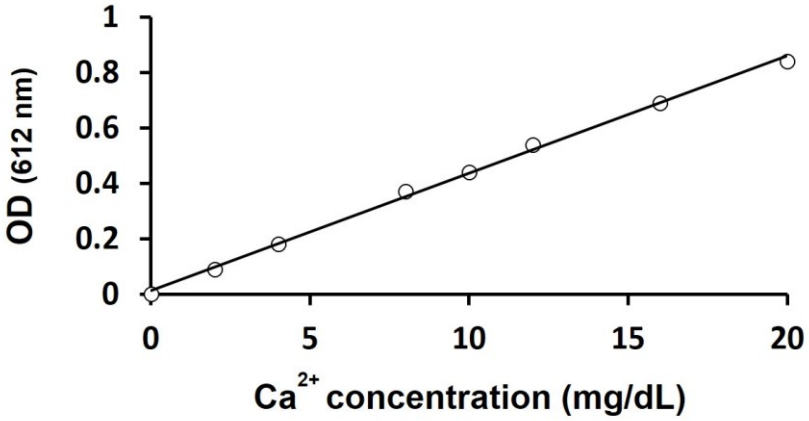
   - OD_{SAMPLE}, OD_{BLANK}, $OD_{STANDARD}$: the O.D. 612 nm values of the sample, sample blank and sample plus standard.
   - 10: the concentration of the standard in mg/dL, and n is the sample dilution factor.
   - If the calculated calcium concentration is greater than 10 mg/dL, dilute sample in distilled water and repeat assay. Multiply result by the dilution factor n.

3. arigo provides GainData®, an in-house development ELISA data calculator, for slope analysis. The slope data can easily be assayed when select regression model as “linear” in GainData®. Please refer our GainData® website for details. (https://www.arigobio.com/elisa-analysis)

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

EXAMPLE OF TYPICAL STANDARD CURVE

The following figures demonstrate typical results with the Calcium Assay Kit (Colorimetric). One should use the data below for reference only. This data should not be used to interpret actual results.



QUALITY ASSURANCE

Sensitivity

0.08 mg/dL (20 µM)

Linear detection range

0.08 mg/dL (20µM) to 20 mg/dL (5mM) Ca^{2+} in 96-well plate assay.