Calcium Assay Kit (Colorimetric)

LS-K211-500 (500 Tests) • **Store at 4°C**

Introduction

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. This 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.

Key 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. Cuvette 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.

Applications

- 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.

Components

Component	K211-500
	500 Tests
Reagent A	50 mL
Reagent B	50 mL
Calcium Standard (20 mg/dL Ca^{2+})	1 mL

Materials 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: Cuvettes and Spectrophotometer for measuring OD_{612nm}.

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Storage

The kit is shipped at room temperature. Store Reagent and Standard at 4°C. Shelf life: 12 months after receipt.

Assay Procedure

Matrix in certain samples (e.g. whole blood) may interfere with the assay. For internal standard protocols, please see **Appendix A** below.

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.

No	STD + H_2O	Vol (µL)	Ca (mg/dL)
1	100µL + 0µL	100	20
2	80µL + 20µL	100	16
3	60µL + 40µL	100	12
4	40µL + 60µL	100	8
5	30µL + 70µL	100	6
6	20µL + 80µL	100	4
7	10µL + 90µL	100	2
8	0µL + 100µL	100	0

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 cuvette and read optical density at 612nm.

Calculations

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

$$= \frac{OD_{SAMPLE} - OD_{BLANK}}{Slope} \quad (mg/dL)$$

OD_{SAMPLE} and OD_{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.

General Considerations

EDTA and other Ca^{2+} chelators interfere with this assay. This assay cannot be applied to plasma samples obtained with EDTA.

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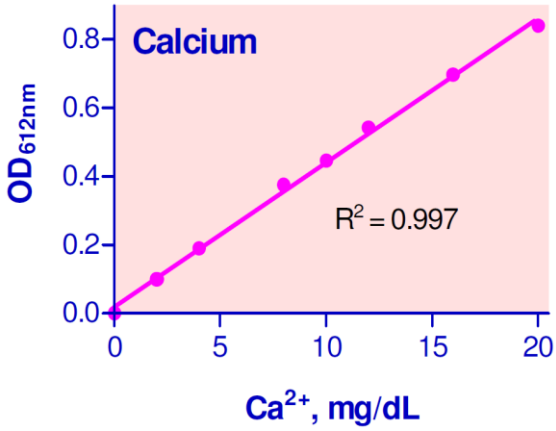
Sample Data

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 fetalbovine 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

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Appendix A

To correct for interference in the sample matrix when using whole blood samples, 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, or is available for purchase upon request.

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_2O.
    a. 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_2O 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. B1. Dilute standard to 10 mg/dL Ca^{2+} by mixing 125 µL 20 mg/dL Standard and 125 µL dH_2O.
2. B2. Transfer 5 µL whole blood sample to a well.
3. B3. Add 200 µL Working Reagent and tap lightly to mix. Note: If any particulates are seen pipette up and down to dissolve.
4. B4. Incubate 3 min at room temperature and read optical density at 570-650 nm (peak absorbance at 612 nm). OD_{SAMPLE}
5. B5. Carefully transfer 5 µL of 10 mg/dL standard to the sample well from step 2. Tap plate to mix. Repeat Step 4. $OD_{STANDARD}$
6. B6. Add 5 µL of 20 mM EDTA to the same well from step 2. Tap plate to mix. Repeat step 4. OD_{BLANK}

Calculations

The whole blood sample concentration is computed as follows:

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

where OD_{SAMPLE}, OD_{BLANK}, and $OD_{STANDARD}$ are the OD readings of the Sample, Sample Blank, and the 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_2O 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.

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