Version 1 Last updated 12 May 2020

ab272527
Calcium Assay Kit

View ab272527
Calcium Assay Kit datasheet:
www.abcam.com/ab272527
(use www.abcam.cn/ab272527 for China, or www.abcam.co.jp/ab272527 for Japan)

For quantitative determination of Calcium in biological, food and environmental samples.

This product is for research use only and is not intended for diagnostic use.

Table of Contents

1. Overview

Calcium Assay Kit (ab272527) is a simple, direct and automation-ready procedure for measuring calcium concentration in biological, environmental and food sample. The 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.

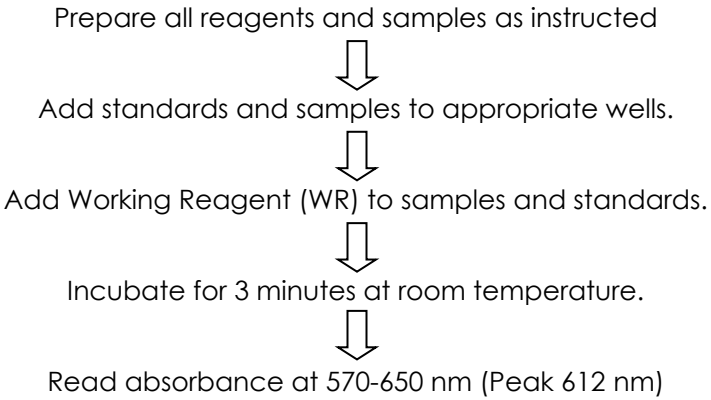
Sensitive and accurate. Use as little as 5 µL samples. Linear detection range 0.08 mg/dL (20 µM) to 20 mg/dL (5 mM) Ca^{2+} in 96-well plate assay.

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.

2. Protocol Summary



3. Precautions

Please read these instructions carefully prior to beginning the assay.

- All kit components have been formulated and quality control tested to function successfully as a kit.
- We understand that, occasionally, experimental protocols might need to be modified to meet unique experimental circumstances. However, we cannot guarantee the performance of the product outside the conditions detailed in this protocol booklet.

Reagents should be treated as possible mutagens and should be handled with care and disposed of properly. Please review the Safety Datasheet (SDS) provided with the product for information on the specific components.

- Observe good laboratory practices. Gloves, lab coat, and protective eyewear should always be worn. Never pipet by mouth. Do not eat, drink or smoke in the laboratory areas.
- All biological materials should be treated as potentially hazardous and handled as such. They should be disposed of in accordance with established safety procedures.

4. Storage and Stability

Store kit at 4°C immediately upon. Avoid multiple freeze-thaw cycles. Kit has a storage time of 12 months from receipt, providing components have not been reconstituted.

Refer to list of materials supplied for storage conditions of individual components. Observe the storage conditions for individual prepared components in the Materials Supplied section.

5. Limitations

- Assay kit intended for research use only. Not for use in diagnostic procedures.
- Do not mix or substitute reagents or materials from other kit lots or vendors. Kits are QC tested as a set of components and performance cannot be guaranteed if utilized separately or substituted.

6. Materials Supplied

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

7. Materials Required, Not Supplied

These materials are not included in the kit, but will be required to successfully perform this assay:

- Pipetting devices and accessories (e.g. 5 µL).
- 96-well clear plate with flat bottom (alternatively, 1 mL cuvettes may be used)
- Standard microplate reader - capable of reading absorbance at 570- 650nm (peak absorbance at 612nm).

8. Technical Hints

- This kit is sold based on number of tests. A 'test' simply refers to a single assay well. The number of wells that contain sample, control or standard will vary by product. Review the protocol completely to confirm this kit meets your requirements. Please contact our Technical Support staff with any questions.
- Pre-rinse the pipette tip with the reagent, use fresh pipette tips for each sample, standard and reagent.
- Pipette standards and samples to the bottom of the wells.
- Add the reagents to the side of the tube to avoid contamination.
- Some Solutions supplied in this kit are caustic; care should be taken with their use.

9. Reagent Preparation

- Equilibrate all reagents to room temperature (18-25°C) prior to use.
- The kit contains enough reagents for 500 assays.

All reagents are supplied ready to use.

10. Standard Preparation

- Always prepare a fresh set of standards for every use.
- Prepare serially diluted standards immediately prior to use.

10.1 Dilute standards as follows. Store diluted standards at 4°C for future use.

Standard #	STD (µL)	H_2O (µL)	Ca^{2+} (mg/dL)
1	100	0	20
2	80	20	16
3	60	40	12
4	40	60	8
5	30	70	6
6	20	80	4
7	10	90	2
8	0	100	0

11. Sample Preparation

Matrix in certain samples (e.g. whole blood) may interfere with the assay.

Whole blood samples require an internal standard (see Section 12).

12. Assay Procedure

- Equilibrate all materials and prepared reagents to room temperature prior to use.
- We recommend that you assay all standards, controls and samples in duplicate.

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

12.1 96-well plate procedure:

12.1.1 Transfer 5 µL diluted standards and samples into wells of a clear bottom 96-well plate.

12.1.2 Prepare enough working reagent by combining equal volumes of Reagent A and B. Add 200 µL working reagent and tap lightly to mix.

Component	Working Reagent (µL/reaction)
Reagent A	100
Reagent B	100

12.1.3 Incubate 3 min at room temperature and read optical density at 570- 650nm (peak absorbance at 612nm).

12.2 Cuvette procedure:

12.2.1 Set up test tubes for diluted standards and Samples. Transfer 15 µL diluted Standards and samples to appropriately labeled tubes.

12.2.2 Add 1000 µL working reagent and vortex to mix. Incubate 3 min. Transfer to cuvette and read optical density at 612nm.

12.3 Assaying Whole Blood:

Protocol A (3 wells required for each sample):

12.3.1 Whole Blood samples require an internal standard and needs 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.

12.3.2 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.

12.3.3 Add 200 µL Working Reagent and tap lightly to mix.
Δ Note: If any particulates or turbidity are seen pipette up and down to dissolve.

12.3.4 Incubate 3 min at room temperature and read optical density at 570-650 nm (peak absorbance at 612 nm).

Protocol B (1 well required for each sample):

12.3.5 Dilute standard to 10 mg/dL Ca^{2+} by mixing 125 µL 20 mg/dL Standard and 125 µL dH_2O.

12.3.6 Transfer 5 µL whole blood sample to a well.

12.3.7 Add 200 µL Working Reagent and tap lightly to mix. Note: If any particulates are seen pipette up and down to dissolve.

12.3.8 Incubate 3 min at room temperature and read optical density at 570-650 nm (peak absorbance at 612 nm). OD_{SAMPLE}

12.3.9 Carefully transfer 5 µL of 10 mg/dL standard to the sample well from step 2. Tap plate to mix. Repeat Step 12.3.8. $OD_{STANDARD}$

12.3.10 Add 5 µL of 20 mM EDTA to the same well from step 2. Tap plate to mix. Repeat step 4. $OD_{BLANK.}$

13. Calculations

13.1 Normal calculation:

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

$$[\text{Calcium}] \text{ in mg/dL} = (OD_{Sample} - OD_{Blank}) / \text{Slope}$$

OD_{SAMPLE} = Sample OD 612 nm value
OD_{BLANK} = Blank OD 612 nm (water or buffer in which the sample was diluted).

Conversion: 1 mg/dL Ca2+ equals 250 µM, 0.001% or 10 ppm.

13.2 Whole blood calculation:

$$[\text{Calcium}] \text{ in mg/dL} = \frac{OD_{SAMPLE} - OD_{BLANK}}{OD_{STANDARD} - OD_{SAMPLE}} \times 10 \times n$$

OD_{SAMPLE} = Sample OD 612 nm value
OD_{BLANK} = Blank OD 612 nm
$OD_{STANDARD}$ = Sample + Standard OD 612 value
10 = Standard concentration in mg/dL

Δ Note: 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.

14. Typical Data

Typical standard curve – data provided **for demonstration purposes only.** A new standard curve must be generated for each assay performed.

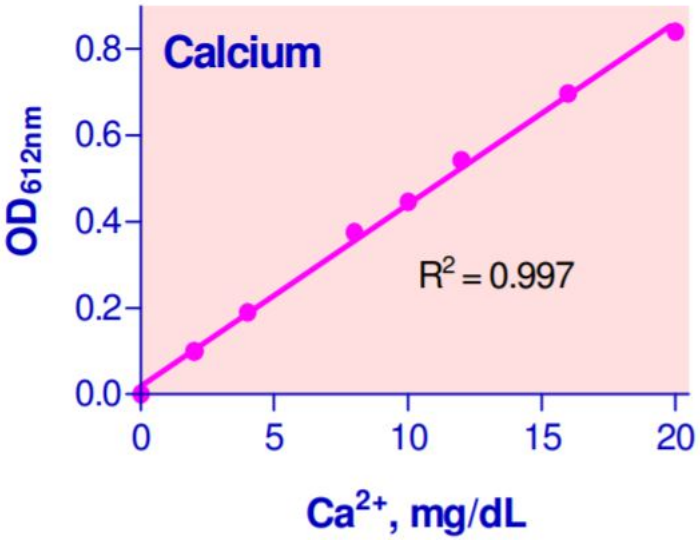


Figure 1. Example of Calcium Assay Kit Ca^{2+} standard curve.

15. Notes

Technical Support

For all technical or commercial enquiries please go to:

www.abcam.com/contactus

www.abcam.cn/contactus (China)

www.abcam.co.jp/contactus (Japan)