Calcium Assay Kit

Catalog Number KA4081

200 assays

Version: 05

Intended for research use only

www.abnova.com

Table of Contents

Introduction 3

Background 3

Principle of the Assay 3

General Information 4

Materials Supplied 4

Storage Instruction 4

Materials Required but Not Supplied 4

Precautions for Use 4

Assay Protocol 5

Reagent Preparation 5

Assay Procedure 5

Example Data Analysis and Figures 6

Resources 7

Plate Layout 7

Introduction

Background

Calcium is essential for all living organisms, particularly in cell physiology, where movement of the calcium ion Ca^{2+} into and out of the cytoplasm functions as a signal for many cellular processes. Calcium is the fifth most abundant element by mass in the human body, where it is a common cellular ionic messenger with many functions, and serves also as a structural element in bone. Calcium plays an important role in mediating the constriction and relaxation of blood vessels, nerve impulse transmission, muscle contraction, and hormone secretion. The serum level of calcium is closely regulated within a fairly limited range (9 to 10.5 mg/dL) in the human body. Both hypocalcaemia and hypercal caemia are serious medical disorders. Causes of low calcium levels include chronic kidney failure, vitamin D deficiency, and low blood magnesium levels that can occur in severe alcoholism.

Principle of the Assay

The Calcium Assay Kit provides a simple method for detecting calcium in physiology solutions. This kit uses our Calcium Blue as the chromogenic calcium indicator. Its absorbance changes in response to calcium binding. Calcium Blue binds calcium tightly in the neutral pH range, generating Calcium Blue-calcium complex that has intense absorption at ~650 nm.

General Information

Materials Supplied

List of component

Component	Amount
Component A: Calcium Blue	10 mL
Component B: Dilution Buffer	20 mL
Component C: 300 mM Calcium Standard	250 µL

Storage Instruction

Component	Storage
Component A: Calcium Blue	Freeze (<-15 °C). Minimize light exposure.
Component B: Dilution Buffer	Freeze (<-15 °C). Minimize light exposure.
Component C: 300 mM Calcium Standard	Freeze (<-15 °C). Minimize light exposure.

Materials Required but Not Supplied

Instrument: Absorbance microplate reader

Absorbance: 600 or 650 nm

Recommended plate: Clear bottom

Precautions for Use

For research use only.

Assay Protocol

Reagent Preparation

✓ Preparation of stock solutions

Unless otherwise noted, all unused stock solutions should be divided into single-use aliquots and stored at -20 °C after preparation. Avoid repeated freeze-thaw cycles.

- Calcium standard solution (3 mM):

  Add 10 µL of Calcium Standard (300 mM) (Component C) to 990 µL Dilution Buffer (Component B) to get Calcium standard solution (3 mM) and mix well.

Assay Procedure

Table 1: Reagent composition for each well

Calcium Standard	Blank Control	Test Sample
Serial dilutions: 50 µL	Dilution Buffer (Component B): 50 µL	50 µL

✓ Calcium assay

1. Add the serially diluted calcium standards from 150 µM to 2.34 µM into wells from CS1 to CS7 in duplicate.
2. Add 50 µL of Calcium Blue (Component A) to each well of calcium standards, blank control, and test samples to make the total calcium assay volume to 100 µL/well.

   Note: For a 384-well plate, add 25 µL of sample and 25 µL of assay reaction mixture into each well.
3. Incubate the reaction for 5 to 10 minutes at room temperature, protected from light.
4. Monitor the absorbance intensity with an absorbance plate reader at OD 600 nm or 650 nm.

✓ Assay Protocol for Serum and Urine Samples

1. Take 10 µL of 300 mM Calcium Standard solution (Component C) to 990 µL Dilution Buffer (Component B) to get 3 mM Calcium Standard Solution.
2. Take 500 µL of 3 mM Calcium Standard Solution to perform 1:2 serial dilutions to get 1.5, 0.75, 0.375, 0.1875, 0.094, 0.047 and 0 mM serially diluted Calcium Standards.
3. Add 10 µL of calcium standard, serum or urine samples and blank control into their respective wells.
4. Add 200 µL of calcium blue (Component A) to each well of calcium standard, blank control, and test samples to make the total calcium assay volume of 210 µL/well.

   Note: For a 384-well plate, add 2.5 µL of sample and 50 µL of assay reaction mixture into each well.
5. Incubate the reactions for 5 – 10 minutes at room temperature (protected from light).
6. Measure the absorbance intensities at 600 nm or 650 nm.

✓ Summary

1. Prepare test samples and calcium standard solution (50 µL)
2. Add Calcium Blue reagent (50 µL)
3. Incubate at room temperature for 5-10 minutes
4. Monitor absorbance intensity at 600 or 650 nm

Important: Thaw all the kit components at room temperature before starting the experiment.

Data Analysis

Example Data Analysis and Figures

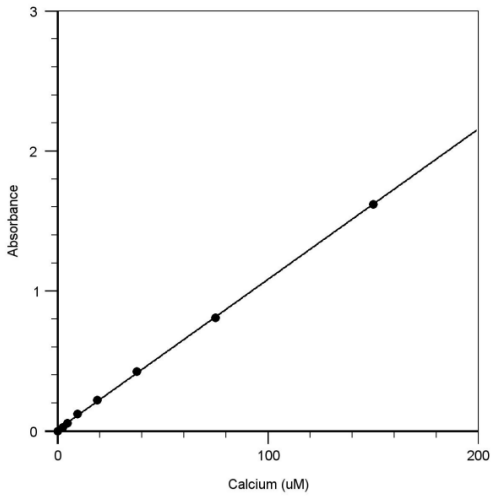

Figure1. Calcium dose response was measured on a 96-well black wall/clear bottom plate with the Calcium Assay Kit. As low as ~ 2.5 µM Ca2+ was detected with 5 minutes incubation time (n=3)

Resources

Plate Layout

	1	2	3	4	5	6	7	8	9	10	11	12
A	Blank Control	Blank Control	Test Samples	Test Samples								
B	Calcium Standards 1	Calcium Standards 1										
C	Calcium Standards 2	Calcium Standards 2										
D	Calcium Standards 3	Calcium Standards 3										
E	Calcium Standards 4	Calcium Standards 4										
F	Calcium Standards 5	Calcium Standards 5										
G	Calcium Standards 6	Calcium Standards 6										
H	Calcium Standards 7	Calcium Standards 7										