Amplite™ Colorimetric Calcium Quantitation Kit *Blue Color*

Catalog number: 36361
Unit size: 200 Tests

Component	Storage	Amount
Component A: Calcium Blue™	Freeze (< -15 °C), Minimize light exposure	1 bottle (10 mL)
Component B: Dilution Buffer	Freeze (< -15 °C), Minimize light exposure	1 bottle (20 mL)
Component C: 300 mM Calcium Standard	Freeze (< -15 °C), Minimize light exposure	1 vial (250 µL)

OVERVIEW

Calcium is essential for all living organisms, particularly in cell physiology, where movement of the calcium ion Ca2+ 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. Amplite™ Calcium Detection 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.

AT A GLANCE

Protocol 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.

KEY PARAMETERS

Absorbance microplate reader

Absorbance	600 or 650 nm
Recommended plate	Clear bottom

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.

PREPARATION OF STANDARD SOLUTION

For convenience, use the Serial Dilution Planner:
https://www.aatbio.com/tools/serial-dilution/36361

Calcium standard

SAMPLE EXPERIMENTAL PROTOCOL

Table 1. Layout of calcium standards and test samples in a clear bottom 96-well microplate. CS = Calcium standard (CS1-CS7); BL = blank control; TS = test sample.

BL	BL	TS	TS
CS1	CS1	...	...
CS2	CS2	...	...
CS3	CS3		
CS4	CS4		
CS5	CS5		
CS6	CS6		
CS7	CS7		

Table 2. Reagent composition for each well

Well	Volume	Reagent
CS1 - CS7	50 µL	Serial Dilutions
BL	50 µL	Dilution Buffer (Component B)
TS	50 µL	test sample

Table 3.

Calcium Standard	Blank Control	Serum or Urine
Serial Dilutions: 50 µL	Dilution Buffer (Compound 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 **.**

EXAMPLE DATA ANALYSIS AND FIGURES

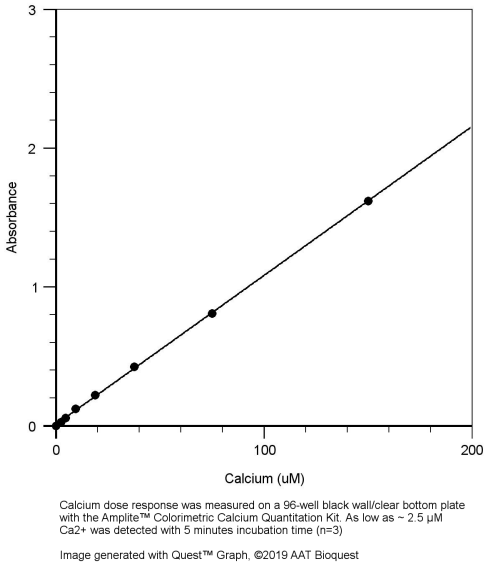

Figure 1. Calcium dose response was measured on a 96-well black wall/clear bottom plate with the Amplite™ Colorimetric Calcium Quantitation Kit. As low as ~ 2.5 µM Ca2+ was detected with 5 minutes incubation time (n=3)

DISCLAIMER

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