Amplite™ Fluorimetric Calcium Quantitation Kit *Red Fluorescence*

Catalog number: 36360
Unit size: 200 Tests

Component	Storage	Amount
Component A: Rhod Red™ Indicator (light sensitive)	Freeze (<-15 °C), Dessicated, Avoid Light	2 vials
Component B: Assay Buffer	Freeze (<-15 °C), Avoid Light	1 bottle (10 mL)
Component C: 300 mM Calcium Standard	Freeze (<-15 °C), Avoid Light	0.5 mL

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 using our proprietary red fluorescence probe provides a simple method for detecting calcium in physiology solutions. The fluorescent signal can be easily read by fluorescence microplate reader with Ex/Em = 540 /590 nm. The Amplite™ Calcium Detection Kit can be performed in a convenient 96-well or 384-well microtiter-plate format and easily adapted to automation with no separation steps required. The assay can be completed within 30 minutes. With the Amplite™ Calcium Detection Kit, we have detected as little as 0.03 mM calcium.

AT A GLANCE

Protocol summary

1. Prepare assay reaction mixture (50 µL)
2. Add calcium standards or test samples (50 µL)
3. Incubate at room temperature for 5 - 30 minutes
4. Monitor the fluorescence intensity at Ex/Em = 540/590 nm

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

KEY PARAMETERS

Instrument:	Fluorescence microplate reader
Excitation:	540 nm
Emission:	590 nm
Cutoff:	570 nm
Recommended plate:	Solid black

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.

1. Rhod Red™ stock solution (200X):
Add 50 µL of sterile H_2O into the vial of Rhod Red™ Indicator (Component A).

PREPARATION OF STANDARD SOLUTION

Calcium standard

For convenience, use the Serial Dilution Planner:
https://www.aatbio.com/tools/serial-dilution/36360

Prepare a calcium standard by diluting the appropriate amount of the 300 mM Calcium Standard (Component C) into H_2O to produce a Calcium concentration ranging from 0 to 3 mM.

PREPARATION OF WORKING SOLUTION

Add 25 µL of Rhod Red™ stock solution (200X) into 5 mL of Assay Buffer (Component B) to make a total volume of 5.025 mL. Keep from light.

SAMPLE EXPERIMENTAL PROTOCOL

Table 1. Layout of calcium standards and test samples in a solid black 96-well microplate. CS= Calcium Standards (CS7 - CS1, 0.003 to 3 mM), BL=Blank Control, TS=Test Samples.

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

Table 2. Reagent composition for each well.

Well	Volume	Reagent
CS1 - CS7	50 µL	Serial Dilutions (0.003 to 3 mM)
BL	50 µL	H_2O
TS	50 µL	test sample

1. Prepare calcium standards (CS), blank controls (BL), and test samples (TS) according to the layout provided in Tables 1 and 2. For a 384-well plate, use 25 µL of reagent per well instead of 50 µL.

2. Add 50 µL of working solution to each well of calcium standard, blank control, and test samples to make the total calcium assay volume of 100 µL/well. For a 384-well plate, add 25 µL of working solution into each well instead, for a total volume of 50 µL/well.

3. Incubate the reaction for 5 to 30 minutes at room temperature, protected from light.

4. Monitor the fluorescence intensity with a fluorescence plate reader at Ex/Em = 540/590 nm.

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EXAMPLE DATA ANALYSIS AND FIGURES

The reading (RFU) obtained from the blank standard well is used as a negative control. Subtract this value from the other standards' readings to obtain the baseline corrected values. Then, plot the standards' readings to obtain a standard curve and equation. This equation can be used to calculate Calcium samples. We recommend using the Online Linear Regression Calculator which can be found at:

https://www.aatbio.com/tools/linear-logarithmic-semi-log-regression-online-calculator/

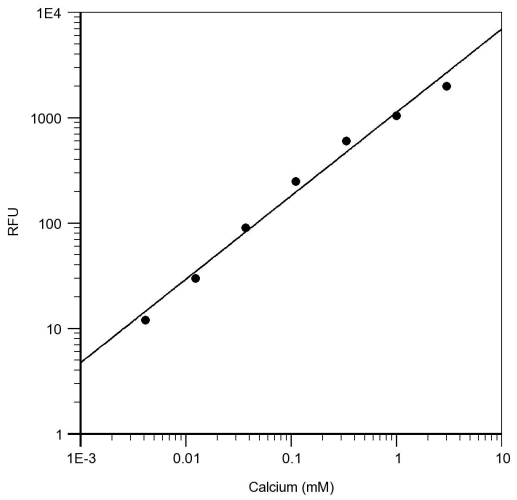

Figure 1. Calcium dose response was measured on a solid black 96-well plate with Amplite™ Fluorimetric Calcium Quantitation Kit.

DISCLAIMER