Amplite™ Fluorimetric Calcium Quantitation Kit

Red Fluorescence

Ordering Information	*Storage Conditions*	*Instrument Platform*
Product Number: 36360 (200 assays)	Keep at -20 °C Avoid exposure to light	Fluorescence microplate readers

Introduction

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 also serves 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 hypocalcemia and hypercalcemia are serious medical disorders. Causes of low calcium levels include chronic kidney failure, vitamin D deficiency, and low blood magnesium levels.

Amplite™ Calcium Quantitation Kit provides a simple method for detecting calcium in physiology solutions by using our proprietary red fluorescence probe. The fluorescence signal can be easily read by a fluorescence microplate reader at Ex/Em = 540/590 nm. The kit can be performed in a convenient 96-well or 384-well microtiter-plate format and easily adapted to automation without a separation step. The assay can be completed within 30 minutes. With the Amplite™ Calcium Quantitation Kit, we have detected as little as 0.03 mM calcium. The kit has a broad dynamic range (30 µM to 10 mM). If more sensitive calcium detection is required, we recommend that Quest Fluo-8™ or Fluo-3 be used instead. Both Quest Fluo-8™ and Fluo-3 can be used for determining calcium in nM range.

Kit Key Features

Sensitive:	Detect as low as 0.03 mM calcium in solution.
Continuous:	Easily adapted to automation without a separation step.
Convenient:	Formulated to have minimal hands-on time. No interference with magnesium.
Non-Radioactive:	No special requirements for waste treatment.

Kit Components

Components	Amount
Component A: Rhod Red™ Indicator (light sensitive)	2 vials
Component B: Assay Buffer	1 bottle (10 mL)
Component C: 300 mM Calcium Standard	0.5 mL

Assay Protocol for One 96-Well Plate

Brief Summary

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

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

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1. Prepare stock solutions:

Prepare 200X Rhod Red™ stock solution by adding 50 µL of sterile H_2O into the vial of Rhod Red™ Indicator (Component A). The stock solution should be used promptly. Any remaining solution needs to be aliquoted and refrozen at -20 ^oC.

2. Prepare assay reaction mixture:

Prepare assay reaction mixture according to the following table, kept from light.

Table 1. Assay reaction mixture for one 96-well plate

Components	**Volume**
Rhod Red™ stock solution (200X, from Step 1)	25 µL
Assay Buffer (Component B)	5 mL
Total Volume	5.025 mL

Table 2. Layout of calcium standards and test samples in a solid black 96-well microplate

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

Note: CS= Calcium Standards, BL=Blank Control, TS=Test Samples.

Table 3. Reagent composition for each well

Ethanol Standard	**Blank Control**	**Test Sample**
Serial dilutions*: 50 µL	H_2O: 50 µL	50 µL

**Note: Add the serially diluted calcium standards from 3 mM to 0.003mM into wells from CS1 to CS7 in duplicate.*

3. Run Calcium assay:

3.1 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 (12 mg/dL). A 0 mM calcium control is included as blank control. The final calcium concentrations will be two folds lower (i.e., 0 to 1.5 mM) with the addition of assay reaction mixture (See Step 3.3).

3.2 Add 50 µL of serial diluted calcium standard (from Step 3.1) into each well.

3.3 Add 50 µL of assay reaction mixture (from Step 2, Table 1) to each well of calcium standard, blank control, and test samples (see Step 2, Table 3) to make the total calcium assay volume of 100 µL/well.
Note: For a 384-well plate, add 25 µL of sample and 25 µL of assay reaction mixture into each well.

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

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

Data Analysis

The fluorescence in blank wells (with H_2O only) is used as a control, and is subtracted from the values for those wells with calcium reactions. A calcium standard curve is shown in Figure 1.
Note: The fluorescence background increases with time, thus it is important to subtract the fluorescence intensity value of the blank wells for each data point.

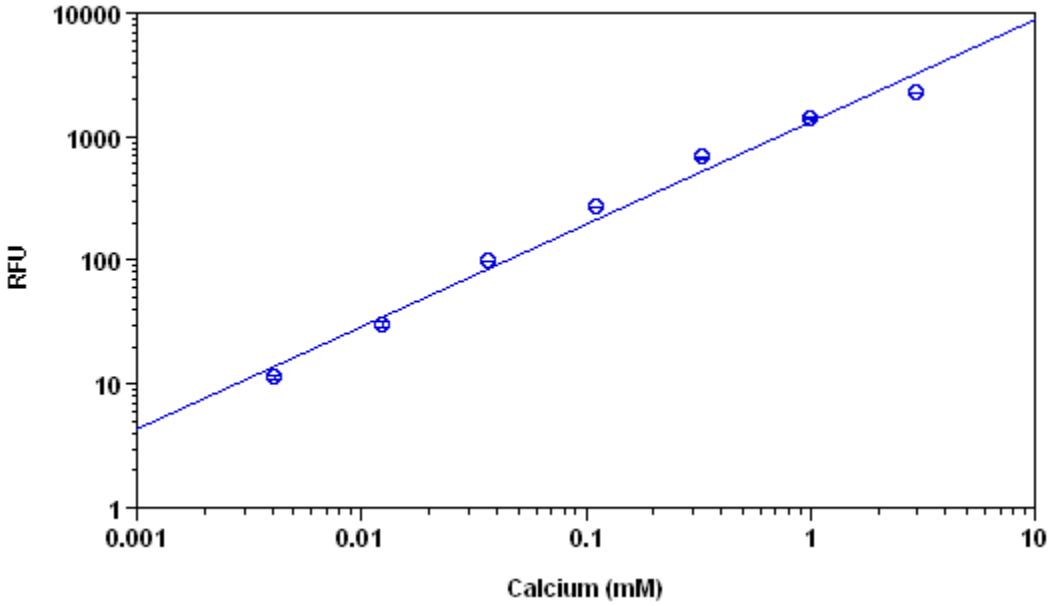

Figure 1. Calcium dose response was measured on a 96-well black plate with the Amplite™ Calcium Quantitation Kit. As low as 0.03 mM calcium can be detected with 5 minutes incubation time (n=3).

References

1. Gangidi RR, Metzger LE. (2006) Ionic calcium determination in skim milk with molecular probes and front-face fluorescence spectroscopy: simple linear regression. J Dairy Sci, 89, 4105.
2. McNamara CJ, Perry TDt, Bearce K, Hernandez-Duque G, Mitchell R. (2005) Measurement of limestone biodeterioration using the Ca2+ binding fluorochrome Rhod- 5N. J Microbiol Methods, 61, 245.
3. David G, Talbot J, Barrett EF. (2003) Quantitative estimate of mitochondrial [Ca2+] in stimulated motor nerve terminals. Cell Calcium, 33, 197.