ab112115

Calcium Quantification Kit - Red Fluorescence

Instructions for Use

For detecting calcium in physiology solutions by using our proprietary red fluorescence probe

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

Version 5d, Last Updated 27 June 2024

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1. 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.

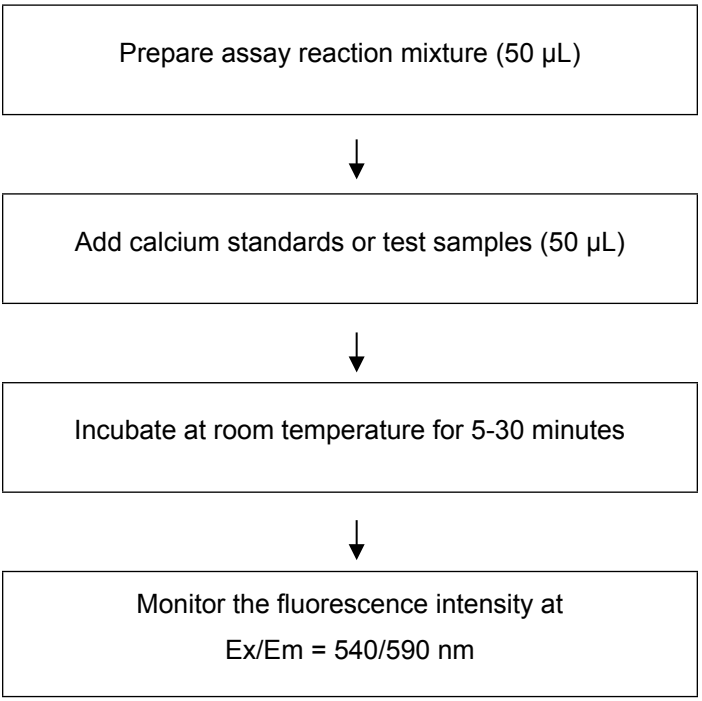
ab112115 provides a simple method for detecting calcium in physiology solutions by using our proprietary red fluorescence probe. The fluorescence signal can be easily read by a fluorescence microplate reader at Ex/Em = 540/590 nm. The kit can be performed in a convenient 96-well or 384-well microtiter-plate format and easily adapted to automation without a separation step. The assay can be completed within 30 minutes. With ab112115, we have detected as little as 0.03 mM calcium. The kit has a broad dynamic range (30 µM to 1 mM).

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.
- Avoid the use of chelators such as EDTA when collecting serum. We recommend using heparin. Mg, Mn, Zn, EGTA, EDTA will interfere with the assay.

2. Protocol Summary

Summary for One 96-well Plate



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

3. Kit Contents

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

4. Storage and Handling

Keep at -20°C. Avoid exposure to light.

5. Assay Protocol

Note: This protocol is for one 96 - well plate.

A. Preparation of Stock Solutions

Prepare 200X Red stock solution by adding 50 µL of sterile H_2O into each 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 °C.

B. Preparation of Assay Reaction Mixture

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

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

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

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

Table 2. Layout of calcium standards and test samples in a solid black 96-well microplate. *Note: CS= Calcium Standards, BL=Blank Control, TS=Test Samples.*

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

Table 3. Reagent composition for each well. **Note: Add the serially diluted calcium standards from 3 mM to 0.003mM into wells from CS1 to CS7 in duplicate.*

C. Run Calcium Assay:

1. Prepare a calcium standard by diluting the appropriate amount of the 300 mM Calcium Standard (Component C) into H2O to produce a calcium concentration ranging from 0 to 6 mM. A 0 mM calcium control is included as blank control. The final calcium concentrations will be two folds lower (i.e., 0 to 3 mM) with the addition of assay reaction mixture (see step 3).

2. Add 50 µL of serial diluted calcium standard (from step 1) into each well.

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 B, 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.

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

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

6. 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.

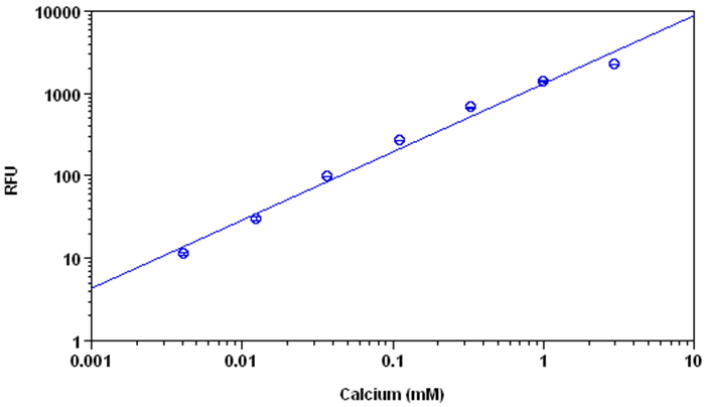


Figure 1. Calcium dose response was measured on a 96-well black plate with ab112115. As low as 0.03 mM calcium can be detected with 5 minutes incubation time (n=3).

7. Troubleshooting

Problem	Reason	Solution
Assay not working	Assay buffer at wrong temperature	Assay buffer must not be chilled - needs to be at RT
	Protocol step missed	Re-read and follow the protocol exactly
	Plate read at incorrect wavelength	Ensure you are using appropriate reader and filter settings (refer to datasheet)
	Unsuitable microtiter plate for assay	Fluorescence: Black plates (clear bottoms); Luminescence: White plates; Colorimetry: Clear plates. If critical, datasheet will indicate whether to use flat- or U-shaped wells
Unexpected results	Measured at wrong wavelength	Use appropriate reader and filter settings described in datasheet
	Samples contain impeding substances	Troubleshoot and also consider deproteinizing samples
	Unsuitable sample type	Use recommended samples types as listed on the datasheet
	Sample readings are outside linear range	Concentrate/ dilute samples to be in linear range

Samples with inconsistent readings	Unsuitable sample type	Refer to datasheet for details about incompatible samples
	Samples prepared in the wrong buffer	Use the assay buffer provided (or refer to datasheet for instructions)
	Samples not deproteinized (if indicated on datasheet)	Use the ***10kDa spin column (ab93349)*** or ***Deproteinizing sample preparation kit (ab93299)***
	Cell/ tissue samples not sufficiently homogenized	Increase sonication time/ number of strokes with the Dounce homogenizer
	Too many freeze-thaw cycles	Aliquot samples to reduce the number of freeze-thaw cycles
	Samples contain impeding substances	Troubleshoot and also consider deproteinizing samples
	Samples are too old or incorrectly stored	Use freshly made samples and store at recommended temperature until use
Lower/ Higher readings in samples and standards	Not fully thawed kit components	Wait for components to thaw completely and gently mix prior use
	Out-of-date kit or incorrectly stored reagents	Always check expiry date and store kit components as recommended on the datasheet
	Reagents sitting for extended periods on ice	Try to prepare a fresh reaction mix prior to each use
	Incorrect incubation time/ temperature	Refer to datasheet for recommended incubation time and/ or temperature
	Incorrect amounts used	Check pipette is calibrated correctly (always use smallest volume pipette that can pipette entire volume)

Problem	Reason	Solution
Standard curve is not linear	Not fully thawed kit components	Wait for components to thaw completely and gently mix prior use
	Pipetting errors when setting up the standard curve	Try not to pipette too small volumes
	Incorrect pipetting when preparing the reaction mix	Always prepare a master mix
	Air bubbles in wells	Air bubbles will interfere with readings; try to avoid producing air bubbles and always remove bubbles prior to reading plates
	Concentration of standard stock incorrect	Recheck datasheet for recommended concentrations of standard stocks
	Errors in standard curve calculations	Refer to datasheet and re-check the calculations
	Use of other reagents than those provided with the kit	Use fresh components from the same kit

For further technical questions please do not hesitate to contact us by email (technical@abcam.com) or phone (select "*contact us*" on www.abcam.com for the phone number for your region).

Technical Support

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