Calcium (Ca^{2+}) Colorimetric Assay Kit

Introduction

Calcium plays a vital role in the human body, not only as a major component of bones and teeth, but also involved in a series of physiological processes such as muscle contraction, cell adhesion, release of hormones and neurotransmitters, glycogen metabolism, and cell proliferation and differentiation. In plasma, Calcium exists in three forms: free, proteins-bound or complexed with anions. Free Calcium is utilized by the body to maintain physiologic functions. Abnormal calcium may lead to the occurrence of a variety of diseases, so monitoring and maintaining the balance of calcium levels is of great clinical importance for the diagnosis and treatment of related diseases.

The Calcium (Ca^{2+}) Colorimetric Assay Kit provides a convenient, highly sensitive, and highly stable method for the detection of calcium ions using o-cresolphthalein complexone (OCPC). Under alkaline conditions, calcium ions can react with OCPC to form a purple complex, which has a maximum absorbance at 575 nm. And the absorbance is proportional to the calcium concentration.

The kit has a wide linear range, maintaining a good linear relationship in the range of 0.2-2 μg of calcium and accurately detecting as little as 0.1 μg of calcium. Liquid samples can be tested directly, while cell and tissue samples can be tested after simple processing.

Components and Storage

Components \ Size	200 Assays	Storage
Lysis Buffer	50 mL	-20°C
Reaction Buffer	15 mL	-20°C
Chromogenic Solution	15 mL	-20°C away from light
Calcium Standard (500 mM)	0.2 mL	-20°C
Shipping: Blue ice	Shelf life: 1 year	

Protocol

1. Sample preparation

1) For cell samples

a) Thaw Lysis Buffer and mix well in advance.

b) For adherent cells, remove the medium and wash once with PBS. For 6-well plates, add 100–200 µL of Lysis Buffer per well and pipette several times to fully lysis cells. For suspension cells, collect cells by centrifugation at 600 g for 5 min at 4°C, discard the supernatant, add 100-200 µL of Lysis Buffer to cells from one well for 6-well plates, and pipette several times to fully lysis cells.

***Note:** The amount of Lysis Buffer can be adjusted according to the specific experiment, and if the cell density is relatively high, it can be increased appropriately.

c) Centrifuge at 10,000 g for 3-5 min at 4°C. Take the supernatant and place it on ice for later use.

***Note:** Prepared samples need to be tested as soon as possible. If it cannot be detected in time, it can be frozen at -80°C and used within one month.

2) For tissue samples

a) Thaw Lysis Buffer and mix well in advance.

b) Wash the tissue once with PBS and subsequently shear the tissue into small pieces with scissors.

c) Add 100-200 µL of Lysis Buffer per 20 mg of tissue and homogenize on ice until well lysed.

***Note:** The amount of Lysis Buffer can be adjusted according to the specific experiment, and the amount can be increased if the lysis is not sufficient.

d) Centrifuge at 10,000 g for 3-5 min at 4°C. Take the supernatant and place it on ice for later use.

***Note:** Prepared samples need to be tested as soon as possible. If it cannot be detected in time, it can be frozen at -80°C and used within one month.

3) Samples of biological fluids such as serum, plasma, urine, etc

a) Use heparin instead of EDTA when preparing serum, as EDTA binds to calcium ions and can affect the detection.

b) Other fluid samples can be tested directly.

c) It is recommended to dilute the sample appropriately to ensure that the test results will fall within the standard values.

***Note:** Prepared samples need to be tested as soon as possible. If it cannot be detected in time, it can be frozen at -80°C and used within one month.

2. Standard Preparation:

a) Prepare a Calcium Standard (5 mM) standard by diluting 10 µL of Calcium Standard (500 mM) in 990 µL of ddH_2O.

b) Refer to the table below to prepare standards curve dilution, 50 µL of standard per well is required, and each dilution has enough amount of standard to set up at least 2 parallel wells.

Standard #	5 mM Calcium Standard (µL)	ddH_2O (µL)	Calcium (mM)	Calcium (µg/well)

1	0	150	0	0
2	3	147	0.1	0.2
3	6	144	0.2	0.4
4	12	138	0.4	0.8
5	18	132	0.6	1.2
6	24	126	0.8	1.6
7	30	120	1.0	2.0

***Note:** The molecular weight of calcium is 40, and the amount of calcium ions per well can be calculated based on this.

3. Calcium detection

1) Thaw Reaction Buffer and Chromogenic Solution at room temperature in advance.

2) 150 μL of assay solution is required per well, and mix appropriate Reaction Buffer and Chromogenic Solution at a ratio of 1:1 to make the assay solution according to the needs of the experiment.

***Note:** The assay solution needs to be prepared and used immediately, and it is recommended to use it within 30 minutes after preparation.

3) Transfer all standards and samples to 96-well plates with a volume of 50 μL per well. For samples, take 2-50 μL of sample and adjust the volume to 50 μL with ddH_2O. It is also recommended to prepare at least two parallel wells for each sample.

4) Add 150 μL of assay solution to each well and mix well.

5) Incubate at room temperature in the dark for 5-10 min.

6) Measure the absorbance at 575 nm by the microplate reader.

***Note:** The assay must be completed within 30 minutes, as the color reaction will fade slightly over time.

4. Data Analysis

1) Subtract the background value of standard #1 from all standard readings, and then plot the standard curve.

2) Subtract the background value of standard #1 from samples to obtain corrected absorbance. Apply corrected absorbance to the standard curve to get A μg of Hydrogen Peroxide in the sample.

3) Calculate the calcium concentration in the sample

$$C=A\times d\div V\ (\mu g/\mu L) \text{ or } C=A\times d\div V\div 40\ (mol/L)$$

Note: A is the calcium amount (μg) in the sample from the standard curve; d is the dilution factor of the sample; V is the volume of the sample added (μL); 40 is the molecular weight of calcium.

Note

1. The pH of the sample needs to be controlled between 6-8, and too high or too low pH will affect the test results.
2. Substances that can bind calcium ions, such as chelating agents (EDTA, EGTA, etc.), citrate, oxalate, and sodium fluoride, can interfere with the detection of this kit.
3. If the sample concentration is too high or too low to cause the test result to be outside the linear range of the standard curve, the sample volume can be adjusted appropriately so that the test result is within the linear range of the standard curve.
4. Reaction Buffer and Chromogenic Solution are harmful to the human body, so you need to take precautions during experiments.
5. For your safety and health, please wear lab coats and gloves during the experiment.
6. For research use only. Not to be used in clinical diagnostic or clinical trials.

APExBIO Technology
www.apexbt.com
7505 Fannin street, Suite 410, Houston, TX 77054.
Tel: +1-832-696-8203 | Fax: +1-832-641-3177 | Email: info@apexbt.com