Calcium Assay Kit

Catalog No.: abx298871

Size: 96 tests (80 samples)

Detection Range: 0.07 mmol/L -1.2 mmol/L

Sensitivity: 0.07 mmol/L

Storage: Store all components at 2-8°C.

Application: For detection and quantification of Calcium content in serum, plasma, tissue homogenate, cell lysates, urine, cell culture supernatant, and other biological fluids.

Introduction

Calcium (Ca2+) is essential in numerous signaling and cellular processes. Calcium mediates nerve impulse transmission, muscle contraction, hormone secretion, as well as the constriction and relaxation of blood vessels. Serum calcium levels are strictly regulated (maintained with the range of 8.4 mg/dL -11.4 mg/dL), and any variation can have serious effects on these processes.

Abbexa's Calcium Assay Kit is a quick, convenient, and sensitive method for measuring and calculating Calcium (Ca2+) concentration. Ca2+ react with MTB. The product concentrations can be determined by measuring the absorbance at 610 nm, from which the concentration of Calcium (Ca2+) can be calculated.

Kit components

1. 96-well microplate
2. MTB reagent: 10 ml
3. Alkali reagent: 20 ml
4. Clarificant: 1 vial
5. Calcium standard (2.5 mmol/L): 10 ml
6. Plate sealer: 2

Materials Required But Not Provided

1. Microplate reader (610 nm)
2. Deionized water
3. Pipette and pipette tips
4. Vials/tubes
5. Incubator
6. Centrifuge
7. Vortex mixer

Protocol

A. Preparation of samples and reagents

1. Samples

Isolate the test samples soon after collecting and analyze immediately or aliquot and store at -20°C or -80°C for long term storage. Avoid multiple freeze-thaw cycles.

The following sample preparation methods are intended as a guide and may be adjusted as required depending on the specific samples used.

- **Serum:** Samples should be collected into a serum separator tube. Coagulate the serum by leaving the tube undisturbed in a vertical position overnight at 4°C or at room temperature for up to 1 hr. Centrifuge at approximately 2000 × g for 15 mins at 4°C. If a precipitate appears, centrifuge again. Take the supernatant, keep on ice and assay immediately, or aliquot and store at -80°C for up to 1 month.
- **Plasma:** Collect plasma using heparin as the anticoagulant. Centrifuge for 10 mins at 1000-2000 x g at 4°C, within 30 mins of collection. If precipitate appears, centrifuge again. Avoid hemolytic samples. Take the supernatant (avoid taking the middle layer containing white blood cells and platelets), keep on ice and assay immediately, or aliquot and store at -80°C for up to 1 month.
- **Tissue Homogenates:** Weigh the tissue homogenate. For each 1 g of homogenate, add 4 ml deionized water. Homogenize by hand, using a mechanical homogenizer, or by ultrasonication. Centrifuge the homogenate at 10,000 x g at 4°C for 10 min. Collect the supernatant and assay immediately. The protein concentration in the supernatant should be determined separately.
- **Cell lysates:** Collect cells into a centrifuge tube and wash with PBS. Centrifuge at 1000 × g for 10 min and discard the supernatant. Add 300-500 µl deionized water 1 × 10^6 cells, then sonicate in an ice water bath. Centrifuge at 1500 × g at 4 °C for 10 min. Take the supernatant into a new centrifuge tube (while kept on ice) and analyze immediately. The protein concentration in the supernatant should be determined separately.
- **Urine:** Collect fresh urine into a sterile container, then centrifuge at 10,000 × g at 4°C for 15 min. Take the supernatant, keep on ice and assay immediately, or aliquot and store at -80°C for up to 1 month.
- **Cell culture supernatant:** Collect cell culture supernatant into a centrifuge tube, then centrifuge at 10,000 × g at 4°C for 10 min. Take the supernatant into a new centrifuge tube (while kept on ice) and analyze immediately, or aliquot and store at -80°C for up to 1 month.

Samples should not contain detergents such as SDS Tween-20, NP-40 and Triton X-100, or reducing agents such as DTT.

It is recommended to carry out a preliminary experiment to determine the optimal dilution factor of samples before carrying out the formal experiment. Dilute samples into different concentrations with normal saline (0.9% NaCl) or PBS (0.01 M, pH 7.4), then carry out the assay procedure.

The recommended dilution factor for different samples is as follows (for reference only):

Sample Type	Dilution Factor
Dog Serum	2-3
Human Serum	3-6
Mouse Serum	3-6
Human Urine	4-8
20% Animal Tissue Homogenate	1

Note:

- Fresh samples or recently obtained samples are recommended to prevent degradation and denaturalization that may lead to erroneous results.
- Lysis buffers may interfere with the kit. It is therefore recommended to use mechanical lysis methods for cell lysates and tissue homogenates.

2. Reagents

- **Clarificant working solution:** Preheat the Clarificant at 37°C until clarified before use. Prepare immediately before carrying out the assay.
- **Working solution 1:** Mix MTB reagent and Alkali reagent at a ratio of 1:2. Prepare immediately before carrying out the assay for serum/plasma sample and mix fully.
- **Working solution 2:** Mix MTB reagent, Alkali reagent and Clarificant working solution at a ratio of 10:20:1. Prepare immediately before carrying out the assay for tissue/cells sample and mix fully.

B. Assay Procedure

1. **Standard curve preparation:** Label 8 tubes with 0, 0.2, 0.3, 0.4, 0.6, 0.8, 1 and 1.2 mmol/L. Dilute the 2.5 mmol/L Calcium standard solution with deionized water to concentrations of 0.2, 0.3, 0.4, 0.6, 0.8, 1 and 1.2 mmol/L. The deionized water itself serves as the 0 mmol/L (blank) standard.
2. Set the Blank, Standard and Sample wells on the well-plate.
3. Add 10 µl of prepared Standards to the Standard wells.
4. Add 10 µl of Sample to sample wells.
5. Add 250 µl of Working solution 1 (for serum/plasma samples) or Working solution 2 (for tissue/cell samples) to each well.
6. Tap the plate gently to mix. Allow to stand at room temperature for 5 minutes.
7. Measure the OD values at 610 nm with a microplate reader.

C. Calculation of Results

The standard curve can be plotted as the absolute OD_{610} of each standard solution (*y*) vs. the respective concentration of the standard solution (*x*). A linear fit is recommended for the standard curve ($y = ax + b$). The Calcium concentration of the samples can be interpolated from the standard curve.

1. Serum, plasma, and other liquid samples:

$$\text{Calcium (mmol/L)} = \frac{\Delta A_{610} - b}{a} \times f$$

2. Tissues and cell lysate samples:

$$\text{Calcium (mmol/g protein)} = \frac{\Delta A_{610} - b}{a} \times \frac{f}{C_P}$$

where:

ΔA_{610}	OD value of the sample ($OD_{Sample} - OD_{Blank}$)
a	gradient of the standard curve (linear fit)
b	y-intercept of the standard curve (linear fit)
f	dilution factor of the sample before carrying out the assay
C_P	concentration of protein in sample (g protein/L)