Calcium test kit (methyl thymol Blue complexed copper microplate method)

Cat: JYM029CHS

Size: 96T

Storage: 2-8°C, avoid light, valid for 6 mouths

Kit Components

	Component	96T	Storage
Reagent 1	MTB Reagent	10mL	2-8℃, avoid light
Reagent 2	Alkaline Solution	20mL	RT
Reagent 3	Protein Clarifying Agent	1mL	RT
Reagent 4	2.5 mmol/L Calcium Standard Solution	1mL	2-8℃, avoid light

Solution Preparation

1. Preparation of 1 mmol/L Calcium Standard Solution: Dilute the 2.5 mmol/L calcium standard solution 2.5-fold with deionized water (2:3 dilution ratio). Prepare fresh before use.
2. Preparation of Working Solution I: Mix Reagent 1 and Reagent 2 at a volume ratio of 1:2. Prepare fresh before use. (For serum/plasma samples.)
3. Preparation of Working Solution II: Mix Reagent 1, Reagent 2 and Reagent 3 at a volume ratio of 10:20:1 Prepare fresh before use. (For tissue and cell samples.)

Note: Reagent 3 may solidify after storage at low temperature. Simply heat it at 37 ℃ to melt before use.

Introduction

Calcium ions in the sample bind with Methylthymol Blue (MTB) in an alkaline solution to form a blue complex. The calcium content in the sample can be calculated by colorimetric comparison with the identically-treated calcium standard.

Required instruments and reagents

Microplate reader (600-630 nm), 96-well plate (one plate supplied), deionized water, protein assay reagents (for tissue or cell samples, available from our company)

Protocols *(only for reference)*

I. Sample Requirements

Collect and process samples following routine laboratory protocols. Valid sample types include serum, plasma (EDTA anticoagulation is not permitted**), tissue homogenate, cells and culture supernatant.

2. Samples are stable for 3-4 days at 2-8℃, and stable for several months below -20℃.

II. Assay Procedures

1. Protocol for serum/plasma and cell culture medium

(1) Assay Setup Table

Reagent	Blank Well	Standard Well	Sample Well
Deionized water(μL)	10		
1 mmol/L Calcium Standard Solution(μL)		10	
Sample (μL)			10
Working Solution I (μL)	250	250	250

Mix thoroughly, incubate for 5 min at room temperature. Measure OD values of each well with a microplate reader at 610 nm.

(2) Calculation Formula

Calcium concentration in sample (mmol/L) = $(A_{sample}-A_{blank}) \div (A_{standard}-A_{blank}) \times C_{standard} \times N$

$C_{standard}$: concentration of standard solution, 1 mmol/L; N: dilution factor of sample prior to testing.

(3) Calculation Examples

A. Rat serum/plasma sample was diluted 2.5-fold with deionized water, and 10 μL of the diluted sample was taken for assay according to the instructions. Measured OD values: Blank well = 0.2428, Standard well = 0.3918, Sample well = 0.3699. Calcium concentration in serum/plasma (mmol/L) = (0.3699 -0.2428) ÷ (0.3918 - 0.2428) × 1 × 2.5 = 2.1326 mmol/L

B. Canine serum/plasma sample was diluted 2.5-fold with deionized water, and 10 μL of the diluted sample was taken for assay according to the instructions. Measured OD values: Blank well = 0.2428, Standard well = 0.3918, Sample well = 0.3902. Calcium concentration in serum/plasma (mmol/L) = (0.3902 − 0.2428) ÷ (0.3918 − 0.2428) × 1 × 2.5 = 2.4732 mmol/L

C. Cell culture supernatant sample was diluted 2.5-fold with deionized water, and 10 μL of the diluted sample was taken for assay according to the instructions. Measured OD values: Blank well = 0.2428, Standard well = 0.3918, Sample well = 0.3639. Calcium concentration in cell culture supernatant (mmol/L) = (0.3639 − 0.2428) ÷ (0.3918 − 0.2428) × 1 × 2.5 = 2.0319 mmol/L

2. Assay for Tissue or Cell Homogenate

(1) Pretreatment

Tissue samples: Weigh the tissue accurately. Add deionized water at a weight-to-volume ratio of 1 g : 9 mL. Homogenize in an ice-water bath. Centrifuge at 2500 rpm for 10 min. Collect the supernatant of 10 % tissue homogenate for testing

Cell samples: Harvest cells (adherent cells: digest with trypsin or scrape with a cell scraper and transfer to a centrifuge tube; centrifuge at 1000-2000 rpm for 5 min, discard supernatant and keep cell pellet. For suspension cells, transfer directly and centrifuge to collect the pellet). Wash the cell pellet 1-2 times with 0.5-1 mL normal saline, collect cell pellet by centrifugation at 1000-2000 rpm. Resuspend cells in 0.3-0.5 mL deionized water. Lyse cells by sonication or manual grinding. After lysis, centrifuge at 2000 rpm for 10 min, collect supernatant for testing

(2) Assay Setup Table

Reagent	Blank Well	Standard Well	Sample Well
Deionized water(μL)	10		
1 mmol/L Calcium Standard Solution(μL)		10	
Tissue homogenate supernatant(μL)			10
Working Solution II (μL)	250	250	250
Mix thoroughly, incubate for 5 min. Measure OD values of each well using a microplate reader at 610 nm			

(3) Calculation Formulas

Formula 1 (for tissue/cell samples, calculated by protein concentration): Calcium content (mmol/gprot)= $(A_{sample}-A_{blank}) \div (A_{standard}-A_{blank}) \times C_{standard} \div Cpr$

Formula 2 (for tissue samples, calculated by fresh tissue weight): Calcium content (mmol/g tissue)= $(A_{sample}-A_{blank}) \div (A_{standard}-A_{blank}) \times C_{standard} \div (W \div V_{total\ sample})$

Formula 3 (for cell samples, calculated by cell number): Calcium content (mmol/10^4) cells)= $(A_{sample}-A_{blank}) \div (A_{standard}-A_{blank}) \times C_{standard} \div (\text{Cell number} \div V_{total\ sample})$

$C_{standard}$:Concentration of standard solution, in mmol/L;

Cpr: Protein concentration of tissue homogenate, gprot/L (prot refers to protein);

W: Mass of tissue sample, g;

$V_{total\ sample}$:Total volume of homogenate solution during sample homogenization, L。

(4) Calculation Example

10 μL of 10% rat brain tissue supernatant was taken and assayed according to the kit instructions. The OD value of blank well was 0.246, the OD value of standard well was 0.336, and the OD value of test well was 0.271 The protein concentration of 10% rat brain tissue supernatant was determined to be 4.02 gprot/L. The calculation result is shown below:

Calcium content in tissue (mmol/gprot)==（0.271-0.246）÷（0.336-0.246）×1÷4.02=0.069 mmol/g prot

Technical Parameters

Wavelength selection range: 600-630 nm

Linear range: 0-2 mmol/L

Note

1. Calcium contamination should be avoided in the experiment. Disposable 96-well plates are recommended.
2. Severe hemolysis, jaundice or lipemia may interfere with test results
3. Deionized water shall be used as the homogenization medium for tissue homogenate preparation to prevent calcium contamination
4. For determination of calcium ion content in tissues or cells, it is recommended to simultaneously measure the protein concentration of homogenate. Protein concentration can be assayed using methods such as PC0010 (Bradford method), PC0020 (BCA method), and BC3185 (biuret method).
5. Prior to the experiment, read and record the OD values of the empty 96-well plate using a microplate reader. Subtract the corresponding empty-plate OD value from each well's measured value before calculation.
6. The product information is for reference only. If you have any questions, please call 400-968-6088 for consultation.
7. The products are all for scientific research use only. Do not use it for medical, clinical diagnosis or treatment, food and cosmetics, etc. Do not store them in ordinary residential areas.
8. For your safety and health, please wear laboratory clothes, disposable gloves and masks to operate