Instructions for Use

Version: 2.0.1

Revision date: 7-Jul-25



Magnesium Assay Kit

Catalog No.: abx298873

Size: 96 tests

Detection Range: 0.18 mmol/L - 2.5 mmol/L

Sensitivity: 0.18 mmol/L

Storage: Store all components at 4°C. Store the Chromogenic Reagent in the dark.

Application: For detection and quantification of Magnesium concentration in serum and plasma samples.

Introduction

Abbexa's Magnesium Assay Kit is a quick, convenient, and sensitive method for measuring and calculating Magnesium concentration. Magnesium present in samples reacts with Calmagite to produce a compound that has an absorption maximum at 540 nm. The intensity of the color is proportional to the concentration of Magnesium, which can then be calculated.

Kit components

- 1. 96-well microplate
- 2. Alkaline Reagent: 16 ml
- 3. Chromogenic Reagent: 16 ml
- 4. Magnesium Standard (5 mmol/L): 1 ml
- 5. Plate sealer: 2

Materials Required But Not Provided

- 1. Microplate reader (540 nm)
- 2. Double-distilled water
- Normal Saline (0.9% NaCl) or PBS (0.01 M, pH 7.4)
- 4. Pipette and pipette tips
- 5. Vials/tubes
- 6. Incubator or water bath
- 7. Vortex mixer

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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 method is intended as a guide and may be adjusted as required depending on the specific samples used.

• **Serum and Plasma**: Serum and plasma samples can be tested directly. Collect plasma using heparin as an anticoagulant. If not tested immediately, samples can be stored at -80°C for up to 1 month.

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
Human serum	1
Rat serum	1
Mouse serum	1
Porcine serum	1
Chicken serum	1

Notes:

• Fresh samples or recently obtained samples are recommended to prevent degradation that may lead to erroneous results.

2. Reagents

- Equilibrate all reagents, apart from the Reaction Working Solution, to room temperature prior to use.
- Reaction Working Solution: For each well to be tested, prepare 250 µl of Reaction Working Solution. Combine Alkaline Reagent and Chromogenic Reagent in a ratio of 1:1. For example, combine 125 µl of Alkali Reagent with 125 µl of Chromogenic Reagent. Mix fully and leave to stand for 10 minutes. Avoid exposure to light during preparation Store at 4°C in the dark for up to 3 days. Incubate at 37°C for 5 minutes immediately before use.
- Standard Dilutions: Label 8 tubes with 0 mmol/L, 0.5 mmol/L, 1 mmol/L, 1.25 mmol/L, 1.5 mmol/L, 1.75 mmol/L, 2 mmol/L, and 2.5 mmol/L. Prepare Standard Dilutions according to the following dilution scheme:

Standard Dilution (mmol/L)	0	0.5	1.0	1.25	1.5	1.75	2.0	2.5
5 mmol/L Standard (μl)	0	10	20	25	30	35	40	50
Double-distilled water (µI)	100	90	80	75	70	65	60	50

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B. Assay Procedure

- 1. Assign and record microplate well positions for each standard, sample, and control. *It is strongly recommended to prepare all wells in duplicate.*
- 2. Add 2.5 µl of the Standard Dilutions to the standard wells.
- 3. Add $2.5 \mu l$ of sample to the sample wells.
- 4. Add 250 µl of Reaction Working Solution to each well and mix fully.
- 5. Incubate at 37°C for 2 minutes.
- 6. Mix fully and measure the OD of each well with a microplate reader at 540 nm.

C. Calculation of Results

Average the duplicate readings for each standard dilution. Subtract the mean OD value of the blank well from each standard dilution to get the absolute OD values. Plot the standard curve, using the OD of the standard dilutions on the y-axis, and their respective concentrations on the x-axis. The curve should form a straight line, described by the formula y = ax + b. Based on this curve, the concentration of Magnesium in each sample well can be derived with the following formula:

Magnesium Concentration (mmol/L) =
$$\frac{OD_{Sample} - OD_{Blank} - b}{a} \times F$$

where:

OD_{Sample} OD value of sample

OD_{Control} OD value of control

a Gradient of the standard curve (y = ax + b)

b Y-intercept of the standard curve (y = ax + b)

F The dilution factor of sample

Technical Support

For troubleshooting and technical assistance, please contact us at <u>support@abbexa.com</u>.