Technical Manual

Magnesium (Mg) Colorimetric Assay Kit

- Catalogue Code: MAES0123
- Size: 100 Assays
- Research Use Only

1. Key features and Sample Types

Detection method:

Colorimetric method

Specification:

100 Assays

Range:

0.12-2.50 mmol/L

Sensitivity:

0.12 mmol/L

Storage:

2-8°C for 6 months

Expiry:

See Kit Label

Experiment Notes:

This kit is for **research use only.**

Instructions should be strictly followed. Changes of operation may result in unreliable results.

The validity of kit is 6 months.

Do not use components from different batches of kit.

Email: info@assaygenie.com **Web:** www.assaygenie.com

2. Background

Magnesium is an important biological element, which is mainly found in bone, muscle cells, soft tissues, serum and red blood cells. It is involved in the synthesis of nucleic acid and protein, and is a cofactor of various enzymes and transporters. It plays an important role in regulating cardiac excitability, neuromuscular conduction, vasomotor contraction, blood pressure and energy metabolism.

3. Intended Use

The kit can be used to detect concentration of magnesium (Mg) in plasma or serum samples.

4. Detection Principle

The magnesium in the serum reacts with the complexometric indicator (Calmagite) to form the Calmagite-Mg compound. The absorbance of this compound at 540 nm is proportional to the concentration of magnesium in the sample. The concentration of magnesium can be calculated by measuring the OD value at 540 nm.

5. Kit components & storage

Item	Specification	Storage
Alkali Reagent	60 mL × 1 vial	2-8°C, 6 months
Chromogenic Agent	60 mL × 1 vial	2-8°C, 6 months, shading light
Magnesium Standard (5 mmol/L)	1 mL × 2 vials	2-8°C, 6 months

Materials required but not supplied

- Micropipettor
- Incubator
- Centrifuge
- Spectrophotometer (540 nm)
- Tips (10 μL, 200 μL, 1000 μL)
- EP tubes (1.5 mL, 2 mL)
- Double distilled water
- Normal Saline (0.9% NaCl)
- PBS (0.01 M, pH 7.4)

6. Assay Notes:

1. Prepare and store the working solution away from direct sunlight.

2. Plasma samples should be an anticoagulant with heparin.

7. Reagent preparation:

1. Bring all reagents to room temperature before use.

2. The preparation of **working solution:** Mix the alkali reagent and chromogenic agent at the ratio of 1:1 and stand for 10 min to prepare the working solution. Prepare fresh solution before use. The prepared solution can be stored at 2-8°C away from direct sunlight for 3 days.

3. The preparation of **standard solution (1 mmol/L):** Dilute magnesium standard (5 mmol/L) with double distilled water 5 times. The prepared solution can be stored at 2-8°C away from direct sunlight for 3 days.

8. Sample Preparation

Sample requirements: Citrate and EDTA should not be used as anticoagulants.

1. Serum sample:
Collect fresh blood and stand at 25°C for 30 min to clot the blood. Then centrifuge at 2000 g for 15 min at 4°C. Take the serum (which is the upper light yellow clarified liquid layer) and preserve on ice before detection. If not detected on the same day, the serum can be stored at -80°C for a month.

2. Plasma sample:
Take fresh blood into the tube which has anticoagulant (heparin is used as anticoagulant, do not use citrate and EDTA as anticoagulants), centrifuge at 700-1000 g for 10 min at 4°C. Take the plasma (which is the upper light yellow clarified liquid layer, don't take white blood cells and platelets in the middle layer) and preserve on ice before detection. If not detected on the same day, the plasma can be stored at -80°C for a month.

Sample Notes:

The concentration should be determined before preforming the assay. If the sample concentration is not within the range of the standard curve, users must determine the optimal sample dilutions for their particular experiments.

If the sample type is not included in the manual, a preliminary experiment is suggested to verify the validity.

Dilution of Samples:

Large variances in results may be seen when performing pre-experiments. Dilute the sample according to the result of the pre-experiment and the detection range (0.12-2.50 mmol/L).

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
Dog serum	1

Note: The diluent is normal saline (0.9% NaCl) or PBS (0.01 M, pH 7.4).

9. Assay Protocol

Ambient Temperature: 25-30°C

Optimum detection wavelength: 540 nm

10. Operation Steps

The measurement of samples

1. **Blank tube:** Add 1000 µL of working solution to 2 mL EP tube.
   Standard tube: Add 1000 µL of working solution to 2 mL EP tube.
   Sample tube: Add 1000 µL of working solution to 2 mL EP tube.

2. Incubate the tubes at 37°C for 5 min.

3. **Blank tube:** Add 10 µL of double distilled water to blank tube.
   Standard tube: Add 10 µL of 1 mmol/L magnesium standard to standard tube.
   Sample tube: Add 10 µL of sample to sample tube.

4. Mix fully with vortex mixer and incubate the tubes at 37°C for 2 min.

5. Set the spectrophotometer to zero with double distilled water and measure the OD value of each tube at 540 nm with 0.5 cm optical path quartz cuvette.

Operation Table

	Blank tube	Standard tube	Sample tube
Working solution (μL)	1000	1000	1000
Incubation at 37°C for 5 min.			
Double distilled water (μL)	10		
1 mmol/L Mg standard solution (μL)		10	
Sample (μL)			10
Mix fully with vortex mixer and incubate the tubes at 37°C for 2 min. Set the spectrophotometer to zero with double distilled water and measure the OD value of each tube at 540 nm with 0.5 cm optical path quartz cuvette.			

11. Calculations

$$\text{Mg content (mmol/L)} = \frac{\Delta A_1}{\Delta A_2} \times c \times f$$

ΔA_1: OD_{sample}-OD_{blank}

ΔA_2: $OD_{standard}$-OD_{blank}

c: The concentration of standard, 1.0 mmol/L

f: Dilution factor of sample before test

12. Performance Characteristics

Detection Range	0.12-2.50 mmol/L
Sensitivity	0.12 mmol/L
Average recovery rate (%)	95
Average inter-assay CV (%)	9.9
Average intra-assay CV (%)	4.8

Analysis

Take 10 μL of mouse serum, carry out the assay according to the operation table.

The results are as follows:

The average OD value of the sample is 0.413, the average OD value of the blank is 0.309, the average OD value of the standard is 0.392, and the calculation result is:

$$\textbf{Mg content (mmol/L)} = \frac{\textbf{0.413-0.309}}{\textbf{0.392-0.309}} \times \textbf{1.0}$$

$$= \textbf{1.25 (mmol/L)}$$

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Safety Notes

Some of the reagents in the kit contain dangerous substances. Avoid touching skin and clothing.

Wash immediately with plenty of water if touching it carelessly.

All the samples and waste material should be treated according to the relevant rules of laboratory's biosafety.

Before the experiment, read the instructions carefully, and wear gloves and work clothes.

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