Technical Manual

Magnesium (Mg) Colorimetric Assay Kit

- Catalogue Code: MAES0122
- Size: 96T
- Research Use Only

1. Key features and Sample Types

Detection method:

Colorimetric method

Specification:

96T

Range:

0.18-2.50 mmol/L

Sensitivity:

0.18 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.

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	16 mL × 1 vial	2-8°C, 6 months
Chromogenic Agent	16 mL × 1 vial	2-8°C, 6 months, avoid direct sunlight
Magnesium Standard (5 mmol/L)	1 mL × 1 vial	2-8°C, 6 months
Microplate	96 wells	No requirement
Plate Sealer	2 pieces	

Materials required but not supplied

- Micropipettor
- Incubator
- Centrifuge
- Microplate Reader (520-550 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 with avoid direct sunlight.

2. The assay temperature of this method is not required strictly. But it should be kept constant, because the color is sensitive to the temperature.

3. Plasma samples should be 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 fully and stand for 10 min to prepare the working solution. Prepare the fresh solution before use. The prepared solution can be stored at 2-8°C with avoid direct sunlight for 3 days. (**Note:** Incubate the prepared working solution at 37°C for 5 min before use)

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) to preserve it on ice for 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) to preserve it on ice for 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 (0.18-2.50 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

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

Plate Set Up:

	1	2	3	4	5	6	7	8	9	10	11	12
A	A	A	S1	S9	S17	S25	S33	S41	S49	S57	S65	S73
B	B	B	S2	S10	S18	S26	S34	S42	S50	S58	S66	S74
C	C	C	S3	S11	S19	S27	S35	S43	S51	S59	S67	S75
D	D	D	S4	S12	S20	S28	S36	S44	S52	S60	S68	S76
E	E	E	S5	S13	S21	S29	S37	S45	S53	S61	S69	S77
F	F	F	S6	S14	S22	S30	S38	S46	S54	S62	S70	S78
G	G	G	S7	S15	S23	S31	S39	S47	S55	S63	S71	S79
H	H	H	S8	S16	S24	S32	S40	S48	S56	S64	S72	S80

Note: A-H, standard wells; S1-S80, sample wells.

10. Operation Steps

The preparation of standard curve

Dilute magnesium standard (5 mmol/L) with double distilled water to a serial concentration. The recommended dilution gradient is as follows: 0, 0.5, 1, 1.25, 1.5, 1.75, 2, 2.5 mmol/L.

The measurement of samples

1. **Standard well:** Add 2.5 μL of standards with different concentrations to corresponding wells.
   Sample well: Add 2.5 μL of sample to corresponding wells.

2. Add 250 μL of working solution to each well.

3. Incubate at 375 mmol/L for 2 min.

4. Mix fully for 5 s with microplate reader. Measure the OD values of each well at 540 nm with microplate reader.

Operation Table

	Standard well	Sample well
Standards with different concentrations (μL)	2.5	
Sample (μL)		2.5
Working solution (μL)	250	250
Incubate at 37°C for 2 min. Mix fully for 5 s with microplate reader. Measure the OD values of each well at 540 nm with microplate reader.		

11. Calculations

Plot the standard curve by using OD value of standard and correspondent concentration as y-axis and x-axis respectively. Create the standard curve with graph software (or EXCEL). The concentration of the sample can be calculated according to the formula based on the OD value of sample. The standard curve is: y= ax + b.

$$\text{Mg content (mmol/L)} = (\Delta A_{540} - b) \div a \times f$$

y: $OD_{Standard} - OD_{Blank}$.

x: The concentration of standard.

a: The slope of standard curve.

b: The intercept of standard curve.

f: Dilution factor of sample before tested.

ΔA_{540}: $OD_{Sample} - OD_{Blank}$.

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12. Performance Characteristics

Detection Range	0.18-2.50 mmol/L
Sensitivity	0.18 mmol/L
Average recovery rate (%)	98
Average inter-assay CV (%)	7.9
Average intra-assay CV (%)	5.1

Analysis

Take 25 μL of human serum, carry the assay according to the operation table.

The results are as follows:
Standard curve: y = 0.1054 x + 0.0097, the average OD value of the sample is 0.618, the average OD value of the blank is 0.503, and the calculation result is:

Mg content (mmol/L) = (0.618 - 0.503 - 0.0097) ÷ 0.1054 = 1.00 (mmol/L)

Safety Notes

Some of the reagents in the kit contain dangerous substances. Prevent 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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