PRODUCT INFORMATION & MANUAL

Magnesium Assay Kit (Colorimetric)

NBP3-25883

For research use only.
Not for diagnostic or therapeutic procedures.

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Novus kits are guaranteed for 6 months from date of receipt

Magnesium (Mg) Colorimetric Assay Kit

Catalog No: NBP3-25883

Method: Colorimetric method

Specification: 96T (Can detect 80 samples without duplication)

Measuring instrument: Microplate reader

Sensitivity: 0.18 mmol/L

Detection range: 0.18-2.50 mmol/L

Average intra-assay CV (%): 5.1

Average inter-assay CV (%): 7.9

Average recovery rate (%): 98

- ▲ This kit is for research use only.
- ▲ Instructions should be followed strictly, changes of operation may result in unreliable results.
- ▲ Please kindly provide us the lot number (on the outside of the box) of the kit for more efficient service.

General information

▲Intended use

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

▲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.

▲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.

▲Kit components & storage

Item	Component	Specification	Storage
Reagent 1	Alkali Reagent	16 mL × 1 vial	2-8℃ , 12 months
Reagent 2	Chromogenic Agent	16 mL × 1 vial	2-8℃ , 12 months, shading light
Reagent 3	5 mmol/L Magnesium Standard	1 mL × 1 vial	2-8℃ , 12 months
	Microplate	96 wells	No requirement
	Plate Sealer	2 pieces	
Note: The reagents must be stored strictly according to the preservation conditions in the above table. The reagents in different kits cannot be mixed with each other.			

▲Materials prepared by users

Instruments

Microplate reader (520-550 nm), Micropipettor, Centrifuge, Incubator, Vortex mixer

Reagents:

Double distilled water, Normal saline (0.9% NaCl), PBS (0.01 M, pH 7.4)

⚠ Safety data

Some of the reagents in the kit contain dangerous substances. It should be avoided to touch the 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.

⚠ Precautions

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

▲ The key points of the assay

1. Prepare and store the working solution with shading light.
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. The color of reaction solution can be stable for 1 hour.
4. Plasma samples should be anticoagulant with heparin.

Pre-assay preparation

▲ Reagent preparation

The preparation of working solution

Mix the reagent 1 and reagent 2 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℃ with shading light for 3 days. (Note: incubate the prepared working solution at 37℃ for 5 min before use)

▲ Sample preparation

Sample requirements

Citrate and EDTA should not be used as anticoagulants.

Serum:

Collect fresh blood and stand at 25℃ for 30 min to clot the blood. Then centrifuge at 2000 g for 15 min at 4℃ . 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℃ for a month.

Plasma:

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℃ . 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℃ for a month.

▲ Dilution of sample

It is recommended to take 2~3 samples with expected large difference to do pre-experiment before formal experiment and 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).

Assay protocol

▲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.

▲Detailed operating steps

The preparation of standard curve

Dilute 5 mmol/L magnesium standard 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. Reference is as follows:

Number	Standard concentrations (mmol/L)	5 mmol/L Standard (μL)	Double distilled water (μL)
A	0	0	100
B	0.50	10	90
C	1.00	20	80
D	1.25	25	75
E	1.50	30	70
F	1.75	35	65
G	2.00	40	60
H	2.50	50	50

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 37℃ 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.

▲Summary 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℃ for 2 min. Mix fully. Measure the OD values at 540 nm .		

▲Calculation

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.

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

Note:

y: $OD_{Standard}$ – OD_{Blank}(OD_{Blank} is the OD value when the standard concentration is 0).

x: The concentration of standard

a: The slope of standard curve

b: The intercept of standard curve

ΔA_{540}: OD_{Sample} – OD_{Blank}

f: Dilution factor of sample before tested

Appendix I Data

▲Example analysis

Take 2.5 μL of human serum and 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:

$$\text{Mg content (mmol/L)} = \frac{0.618 - 0.503 - 0.0097}{0.1054} = 1.00 \text{ mmol/L}$$

Appendix II References

1. Mooren, C. F. Magnesium and disturbances in carbohydrate metabolism[J]. Diabetes Obesity & Metabolism, 2015, 17(9): 813-823.
2. Won S J, Jin P T. Magnesium Metabolism[J]. Electrolytes & Blood Pressure E & Bp, 2008, 6(2): 86-95.
3. Champagne C M. Magnesium in Hypertension, Cardiovascular Disease, Metabolic Syndrome, and Other Conditions: A Review[J]. Nutrition in Clinical Practice, 2008, 23(2): 142-151.
4. Noronha L J, Matuschak G M. Magnesium in critical illness: metabolism, assessment, and treatment[J]. Intensive Care Med, 2002, 28(6): 667-679.