（FOR RESEARCH USE ONLY. DO NOT USE IT IN CLINICAL DIAGNOSIS !）

Catalog No：E-BC-K162-M

Specification：48T(32 samples)/96T(80 samples)

Measuring instrument：Microplate reader（520-550 nm）

Detection range：0.18-2.50 mmol/L

Elabscience® Magnesium (Mg) Colorimetric Assay Kit

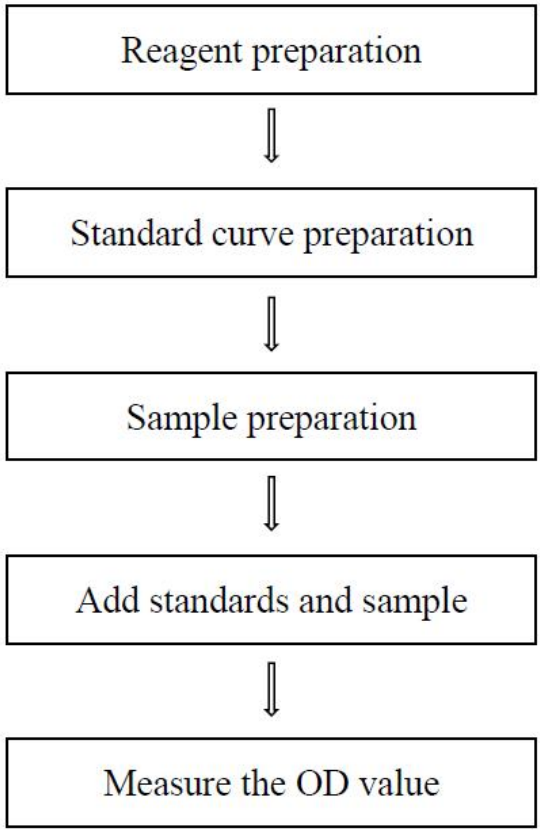
This manual must be read attentively and completely before using this product.
If you have any problem, please contact our Technical Service Center for help：

Toll-free: 1-888-852-8623
Tell: 1-832-243-6086
Fax: 1-832-243-6017
Email: techsupport@elabscience.com
Website: www.elabscience.com

Please kindly provide us the lot number (on the outside of the box) of the kit for more efficient service.

Table of contents

Assay summary



Intended use

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

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	Size 1(48 T)	Size 2(96 T)	Storage
Reagent 1	Alkali Reagent	8 mL × 1 vial	16 mL × 1 vial	2-8°C, 12 months
Reagent 2	Chromogenic Agent	8 mL × 1 vial	16 mL × 1 vial	2-8°C, 12 months, shading light
Reagent 3	5 mmol/L Magnesium Standard	1 mL × 1 vial	1 mL × 1 vial	2-8°C, 12 months
	Microplate	48 wells	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. For a small volume of reagents, please centrifuge before use, so as not to obtain sufficient amount of reagents.

Materials prepared by users

Instruments:

Microplate reader (520-550 nm, optimum wavelength: 540 nm), Micropipettor, Centrifuge, Incubator, Vortex mixer.

Reagents:

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

Reagent preparation

① Equilibrate all the reagents to room temperature before use.

② The preparation of working solution：

For each well, prepare 250 μL of working solution (mix well 125 μL of alkali reagent and 125 μL of chromogenic agent), mix and stand for 10 min to prepare the working solution. The working solution should be prepared on spot. The working solution can be stored at 2-8°C for 3 days protected from light. (Note: incubate the prepared working solution at 37°C for 5 min before use).

③ The preparation of standard curve：

Always prepare a fresh set of standards. Discard working standard dilutions after use.

Dilute 5 mmol/L standard solution with double distilled water diluent 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:

Item	①	②	③	④	⑤	⑥	⑦	⑧
Concentration (mmol/L)	**0**	**0.5**	**1.0**	**1.25**	**1.50**	**1.75**	**2.00**	**2.50**
5 mmol/L standard (μL)	0	10	20	25	30	35	40	50
Double distilled water (μL)	100	90	80	75	70	65	60	50

Sample preparation

① Sample preparation:

Serum and plasma: detect directly. If not detected on the same day, the serum or plasma can be stored at -80°C for a month.

② Dilution of sample

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). For the dilution of other sample types, please do pretest to confirm the dilution factor.

The key points of the assay

① Prepare and store the working solution protected from 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 h.

④ Plasma samples should be anticoagulant with heparin.

Operating steps

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

② Add 250 μL of working solution to each well.

③ Incubate at 37°C 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.

Calculation

The standard curve:

1. Average the duplicate reading for each standard.

2. Subtract the mean OD value of the blank (Standard #①) from all standard readings. This is the absoluted OD value.

3. Plot the standard curve by using absoluted OD value of standard and correspondent concentration as y-axis and x-axis respectively. Create the standard curve (**y = ax + b**) with graph software (or EXCEL).

The sample:

Serum (plasma) sample:

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

[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}: Absolute OD ($OD_{Sample} - OD_{Blank}$).

f: Dilution factor of sample before tested.

Appendix I Performance Characteristics

1. Parameter:

Intra-assay Precision

Three human serum samples were assayed in replicates of 20 to determine precision within an assay. (CV = Coefficient of Variation)

Parameters	Sample 1	Sample 2	Sample 3
Mean (mmol/L)	0.68	1.35	2.20
%CV	5.6	5.1	4.6

Inter-assay Precision

Three human serum samples were assayed 20 times in duplicate by three operators to determine precision between assays.

Parameters	Sample 1	Sample 2	Sample 3
Mean (mmol/L)	0.68	1.35	2.20
%CV	8.2	7.1	8.4

Recovery

Take three samples of high concentration, middle concentration and low concentration to test the samples of each concentration for 6 times parallelly to get the average recovery rate of 98%.

	Standard 1	Standard 2	Standard 3
Expected Conc. (mmol/L)	0.8	1.46	1.95
Observed Conc. (mmol/L)	0.8	1.4	1.9
Recovery rate (%)	101	95	98

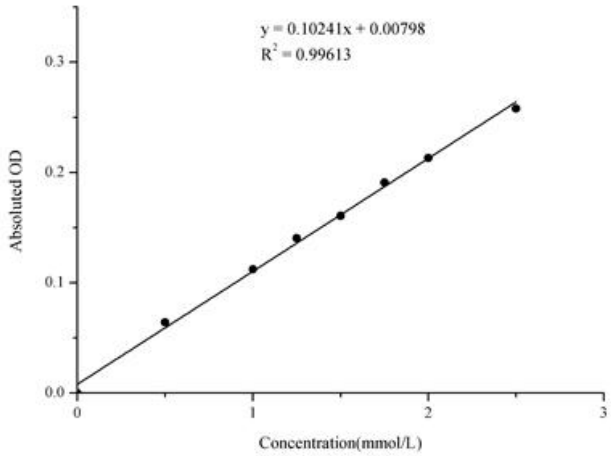
Sensitivity

The analytical sensitivity of the assay is 0.18 mmol/L. This was determined by adding two standard deviations to the mean O.D. obtained when the zero standard was assayed 20 times, and calculating the corresponding concentration.

2. Standard curve

As the OD value of the standard curve may vary according to the conditions of the actual assay performance (e.g. operator, pipetting technique or temperature effects), so the standard curve and data are provided as below for reference only.

Concentration (mmol/L)	0	0.5	1	1.25	1.5	1.75	2	2.5
Average OD	0.450	0.514	0.563	0.591	0.611	0.641	0.663	0.708
Absoluted OD	0	0.064	0.113	0.140	0.161	0.191	0.213	0.258



Appendix II Example Analysis

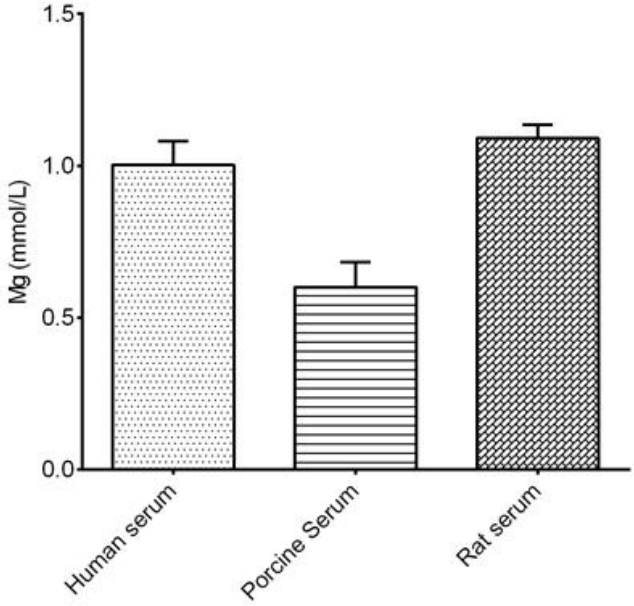
Example analysis:

Take 2.5 μL of human serum and carry the assay according to the operation steps. 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:

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

Detect human serum, porcine serum, rat serum according to the protocol, the result is as follows:



Appendix III Publications

1. Shi Z , Yang F , Hu Y ,et al.An oxidized dextran-composite self-healing coated magnesium scaffold reduces apoptosis to induce bone regeneration[J].Carbohydrate polymers, 327:121666[2025-03-04].DOI:10.1016/j.carbpol.2023.121666.
2. Zeng Z, Quan C, Zhou S, et al. Gut microbiota and metabolic modulation by supplementation of polysaccharide-producing Bacillus licheniformis from Tibetan Yaks: A comprehensive multi-omics analysis[J]. International Journal of Biological Macromolecules, 2024, 254: 127808.
3. Yue Z, Chen Y, Wang Y, et al. Halotolerant Bacillus altitudinis WR10 improves salt tolerance in wheat via a multi-level mechanism[J]. Frontiers in Plant Science, 2022, 13: 941388.
4. Jia R , He Y , Liang J ,et al.Preparation of biocompatibility coating on magnesium alloy surface by sodium alginate and carboxymethyl chitosan hydrogel[J].iScience, 27(3):109197.DOI:10.1016/j.isci.2024.109197.
5. Wu J , Jiao C , Yu H ,et al.A tailored hydroxyapatite/magnesium silicate 3D composite scaffold: Mechanical, degradation, and bioactivity properties[J].Ceramics International, 2023(22 Pt.A):49.DOI:10.1016/j.ceramint.2023.08.221.
6. Hu J , Huang G , Li L ,et al.A Mg2+-light double crosslinked injectable alginate hydrogel promotes vascularized osteogenesis by establishing a Mg2+-enriched microenvironment[J].Materials Today Communications, 2024, 41.DOI:10.1016/j.mtcomm.2024.110303.

Statement

1. This assay kit is for Research Use Only. We will not response for any arising problems or legal responsibilities causing by using the kit for clinical diagnosis or other purpose.
2. Please read the instructions carefully and adjust the instruments before the experiments. Please follow the instructions strictly during the experiments.
3. Protection methods must be taken by wearing lab coat and latex gloves.
4. If the concentration of substance is not within the detection range exactly, an extra dilution or concentration should be taken for the sample.
5. It is recommended to take a pre-test if your sample is not listed in the instruction book.
6. The experimental results are closely related to the situation of reagents, operations, environment and so on. Elabscience will guarantee the quality of the kits only, and NOT be responsible for the sample consumption caused by using the assay kits. It is better to calculate the possible usage of sample and reserve sufficient samples before use.