Catalog No：E-BC-K162-S

Specification：50 assays(48 samples)/100 assays(98 samples)/500 assays(498 samples)

Measuring instrument：Spectrophotometer（540 nm）

Detection range：0.12-2.50 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.

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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 (50 assays)	Size 2 (100 assays)	Size 3 (500 assays)	Storage
Reagent 1	Alkali Reagent	30 mL × 1 vial	60 mL × 1 vial	60 mL × 5 vials	2-8°C, 12 months
Reagent 2	Chromogenic Agent	30 mL × 1 vial	60 mL × 1 vial	60 mL × 5 vials	2-8°C, 12 months, shading light
Reagent 3	5 mmol/L Magnesium Standard	1 mL × 1 vial	1 mL × 2 vials	1 mL × 10 vials	2-8°C, 12 months

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:

Spectrophotometer (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 1000 μL of working solution (mix well 500 μL of alkali reagent and 500 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.

③ The preparation of 1 mmol/L standard solution：

For each well, prepare 10 μL of 1 mmol/L standard solution (mix well 2 μL of 5 mmol/L magnesium standard and 8 μL of double distilled water). Stored at 2-8°C for 3 days.

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
Dog 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 hour.

④ Plasma samples should be anticoagulant with heparin.

Operating steps

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.

Calculation

The sample:

Serum (plasma) sample:

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

[Note]

ΔA_1: $OD_{Sample} - OD_{Blank}$

ΔA_2: $OD_{Standard} - OD_{Blank}$

c: The concentration of standard, 1 mmol/L.

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)	0.55	1.35	2.30
%CV	5.3	4.6	4.5

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)	0.55	1.35	2.30
%CV	10.2	9.4	10.1

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 95%.

	Sample 1	Sample 2	Sample 3
Expected Conc. (mmol/L)	0.35	1.06	1.95
Observed Conc. (mmol/L)	0.3	1.0	1.8
Recovery rate (%)	94	98	93

Sensitivity

The analytical sensitivity of the assay is 0.12 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

(It doesn't need to prepare the standard curve for this kit and the provided standard curve is for reference only)

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	0.75	1	1.5	2	2.5
Average OD	0.331	0.374	0.390	0.410	0.445	0.484	0.514
Absoluted OD	0	0.043	0.059	0.079	0.114	0.153	0.183

$y = 0.07303 x + 0.004$
$R^2 = 0.9978$
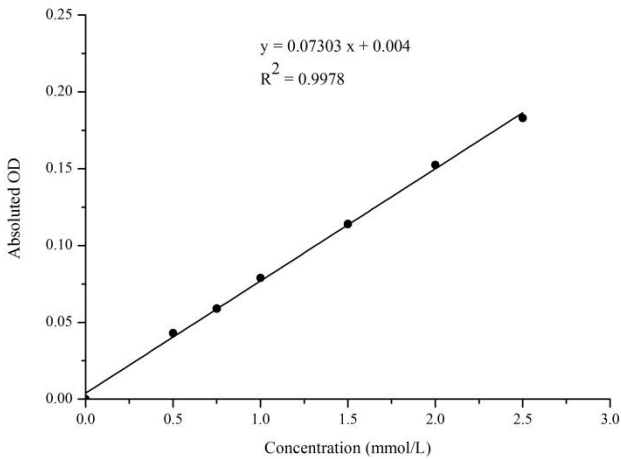
Appendix Ⅱ Example Analysis

Example analysis：

Take 10 μL of mouse serum and carry the assay according to the operation steps. 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:

$$\frac{\text{Mg content}}{\text{(mmol/L)}} = \frac{0.413 - 0.309}{0.392 - 0.309} \times 1.0 = 1.25 \ (\text{mmol/L})$$

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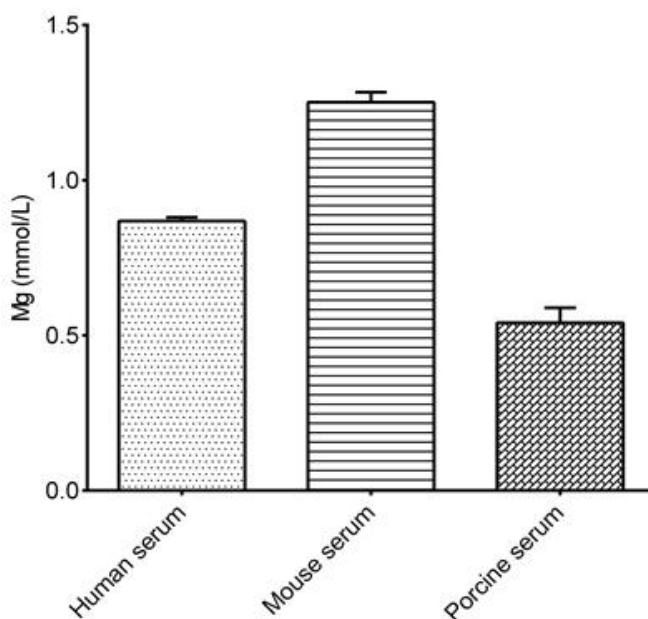
Statement

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.