# TECHNICAL MANUAL

# Magnesium (Mg) Colorimetric Assay Kit

- **SKU CODE:** MAES0122
- **SIZE:** 48 Tests
- **DETECTION PRINCIPLE:** Assay Kit
- **RUO:** Research-Use-Only

Revised: 01/2026

# 1. Assay summary

- Reagent preparation
- Standard curve preparation
- Sample preparation
- Add standards and samples
- Measure OD values at 540 nm

# 2. Intended use

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

# 3. Detection principle

The magnesium in serum reacts with the complexometric indicator (Calmagite) to form a 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.

# 4. 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 | 2 pieces | |

# 5. 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)

# 6. Reagent preparation

1. Equilibrate all reagents to room temperature before use.
2. **Preparation of working solution:** For each well, prepare 250 µL of working solution by mixing 125 µL of alkali reagent with 125 µL of chromogenic agent. Mix well and let stand for 10 minutes to prepare the working solution. The working solution should be prepared fresh. 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 minutes before use).
3. **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 to create a serial concentration. The recommended dilution gradient is: 0, 0.5, 1, 1.25, 1.5, 1.75, 2, 2.5 mmol/L.

# 7. Sample preparation

**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 one month.

**Dilution of sample:**

The recommended dilution factor for different samples is as follows (for reference only):

- 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 dilution of other sample types, please perform a pretest to confirm the dilution factor.

# 8. 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 strictly required, but it should be kept constant because the color is sensitive to temperature.
3. The color of the reaction solution can be stable for 1 hour.
4. Plasma samples should be anticoagulated with heparin.

# 9. 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 minutes.
4. Mix fully for 5 seconds with microplate reader. Measure the OD values of each well at 540 nm with microplate reader.

# 10. 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 absolute OD value.

3. Plot the standard curve using absolute OD value of standard and corresponding 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:

Mg content (mmol/L) = (ΔA_540 - b) ÷ a × 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.

# 11. Appendix I Performance Characteristics

Parameter:

## Intra-assay Precision

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

## Intra-assay Precision

| 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.

## Inter-assay Precision

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

## Recovery

Three samples of high, middle, and low concentration were tested with 6 replicates for each concentration to obtain an average recovery rate of 98%.

## Recovery

| Parameters | Standard 1 | Standard 2 | Standard 3 |
|---|---|---|---|
| Expected Conc. (mmol/L) | 0.8 | 1.46 | 1.95 |

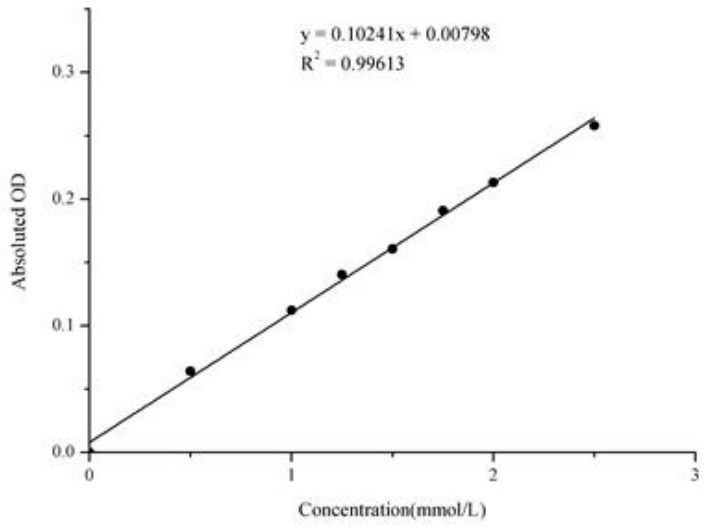
| Parameters | Standard 1 | Standard 2 | Standard 3 |
|---|---|---|---|
| Observed Conc. (mmol/L) | 0.8 | 1.4 | 1.9 |
| Recovery rate (%) | 101 | 95 | 98 |

## 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.

## 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), the standard curve and data are provided below for reference only.




## Standard curve

| Concentration (mmol/L) | Average OD | Absolute OD |
|---|---|---|
| 0 | 0.450 | 0 |
| 0.5 | 0.514 | 0.064 |
| 1 | 0.563 | 0.113 |

| Concentration (mmol/L) | Average OD | Absolute OD |
|---|---|---|
| 1.25 | 0.591 | 0.140 |
| 1.5 | 0.611 | 0.161 |
| 1.75 | 0.641 | 0.191 |
| 2 | 0.663 | 0.213 |
| 2.5 | 0.708 | 0.258 |

# 12. Appendix II Example Analysis

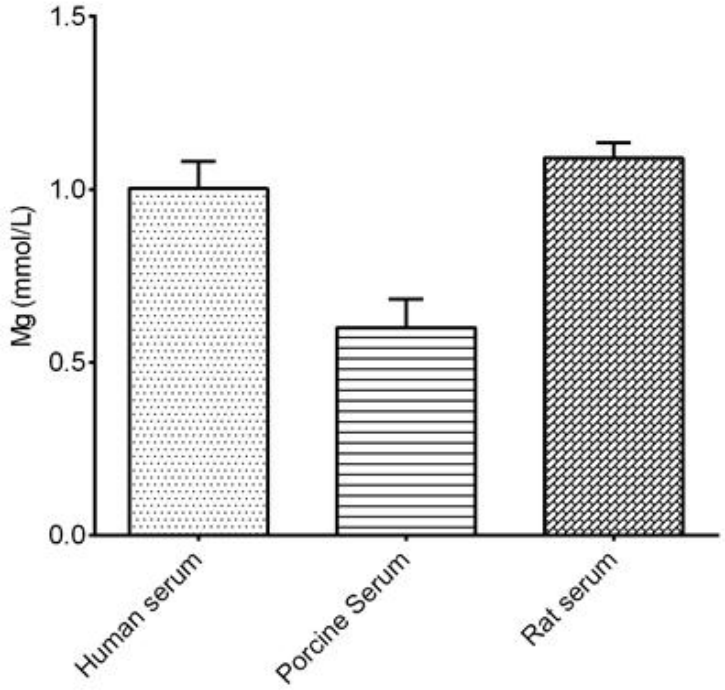
**Example analysis:**

Take 2.5 µL of human serum and carry out the assay according to the operation steps. The results are as follows:

**Standard curve:** y = 0.1054x + 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)

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



# 13. Statement

1. This assay kit is for Research Use Only. Assay Genie assumes no responsibility for any problems or legal liabilities arising from the use of this kit for clinical diagnosis or any other purpose.
2. Please read the instructions carefully and calibrate the instruments before performing the experiments. Follow the instructions strictly throughout the procedure.
3. Appropriate protective measures must be taken, including wearing a lab coat and latex gloves.
4. If the concentration of the substance falls outside the detection range, perform an additional dilution or concentration step on the sample.
5. It is recommended to perform a pre-test if your sample type is not listed in the instruction manual.
6. Experimental results are closely related to reagent quality, operator technique, environmental conditions, and other factors. Assay Genie guarantees the quality of the kits only and is NOT responsible for sample consumption resulting from use of the assay kits. It is advisable to estimate the expected sample usage and reserve sufficient samples before starting the experiment.

**Assay Genie 100% money-back guarantee!**

If you are not satisfied with the quality of our products and our technical team cannot resolve your problem, we will give you 100% of your money back.

**Manufacturers Statement: This final kit system is assembled and quality-released by Assay Genie Limited.**