# Magnesium Assay Kit

**Catalog No.:** abx298873

**Size:** 96 tests

**Detection Range:** 0.18 mmol/L – 2.5 mmol/L

**Sensitivity:** 0.18 mmol/L

**Storage:** Store all components at 4°C in the dark.

**Application:** For detection and quantification of Magnesium in serum and plasma samples.

## Introduction

Abbexa's Magnesium Assay Kit is a quick, convenient, and sensitive method for measuring and calculating Magnesium concentration. Magnesium present in samples reacts with Calmagite to produce a compound that has an absorption maximum at 540 nm. The intensity of yellow color is proportional to the concentration of Magnesium, which can then be calculated.

## Kit components

1. 96-well microplate
2. Alkali Reagent: 16 ml
3. Detection Reagent: 16 ml
4. Magnesium Standard (5 mmol/L): 1 ml
5. Plate sealer: 2

## Materials Required But Not Provided

1. Microplate reader (540 nm)
2. Double distilled water
3. Normal Saline (0.9% NaCl) or PBS (0.01 M, pH 7.4)
4. Pipette and pipette tips
5. Vials/tubes
6. Incubator or Sonicating water bath
7. Vortex mixer

## Protocol

### A. Preparation of samples and reagents

#### 1. Samples

Isolate the test samples soon after collecting and analyze immediately or aliquot and store at -20°C or -80°C for long term storage. Avoid multiple freeze-thaw cycles.

The following sample preparation methods are intended as a guide and may be adjusted as required depending on the specific samples used.

- **Serum:** Collect the serum using a serum separator tube and allow to stand for 1-2 h at room temperature or overnight at 4°C. Centrifuge for 15 min at 2000 × g at 4°C. Transfer the supernatant into a clean tube and analyse immediately. Bring samples to room temperature before carrying out the assay.
- **Plasma**: Collect the plasma in a tube using heparin as an anticoagulant. Centrifuge for 15 min at 1000 × g at 2-8°C within 30 min of collection. Transfer the supernatant into a clean tube and analyse immediately. Bring samples to room temperature before carrying out the assay.

**Notes:**

- **Only use Heparin as the anticoagulant for plasma.** Citrate and EDTA are not suitable for use with this assay.
- Fresh samples or recently obtained samples are recommended to prevent degradation that may lead to erroneous results.
- Samples should not contain detergents such as SDS Tween-20, NP-40 and Triton X-100, or reducing agents such as DTT.

It is recommended to carry out a preliminary experiment to determine the optimal dilution factor of samples before carrying out the formal experiment. Dilute samples into different concentrations with Normal Saline (0.9% NaCl) or PBS (0.01 M, pH 7.4) then carry out the assay procedure.

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 |
| Pig serum | 1 |
| Chicken serum | 1 |

#### 2. Reagents

Bring all reagents to room temperature prior to use.

- **Reaction working solution:** Mix Alkali Reagent and Detection Reagent at a ratio of 1:1 (for example, add 16 ml of Alkali Reagent to 16 ml of Detection Reagent). Mix fully and stand in the dark for 10 minutes. Incubate at 37°C for 5 minutes immediately before use in the assay. ***This solution should be kept in the dark.***

### B. Assay Procedure

1. **Standard curve preparation:** Label 8 tubes with 0, 0.50, 1.00, 1.25, 1.50, 1.75, 2.00 and 2.50 mmol/L. Prepare the standard tubes according to the following table.

| Volume of 5 mmol/L Standard (µl) | Volume of Double Distilled water (µl) | Standard concentration |
|---|---|---|
| 0 | 100 | 0 |
| 10 | 90 | 0.50 |
| 20 | 80 | 1.00 |
| 25 | 75 | 1.25 |
| 30 | 70 | 1.50 |
| 35 | 65 | 1.75 |
| 40 | 60 | 2.00 |
| 50 | 50 | 2.50 |

2. Set standard and sample wells on the microplate and record their positions.
3. Add 2.5 µl of each standard to the standard wells.
4. Add 2.5 µl of each sample to the sample wells.
5. Add 250 µl of Reaction working solution to all wells and mix fully.
6. Incubate at 37°C for 2 minutes.
7. Tap the plate gently to mix. Read and record the absorbance at 540 nm with a microplate reader.

### C. Calculation of Results

The standard curve can be plotted as the absolute $OD_{540}$ of each standard solution ($y$) vs. the respective concentration of the standard solution ($x$). A linear fit is recommended for the standard curve ($y = ax + b$). The Magnesium concentration of the samples can be interpolated from the standard curve.

$$\text{Magnesium (mmol/L)} = \frac{\Delta A - b}{a} \times f$$

where:

| | |
|---|---|
| $\Delta A$ | OD value of the sample ($OD_{Sample} - OD_{Blank}$) |
| $a$ | gradient of the standard curve (linear fit) |
| $b$ | y-intercept of the standard curve (linear fit) |
| $f$ | dilution factor of the sample before carrying out the assay |