Technical Bulletin

Magnesium Assay Kit

Catalogue number MAK507

Product Description

Magnesium (Mg) is one of the most abundant and essential minerals in mammals. Magnesium is involved in more than 300 biochemical reactions in the body and plays important roles in muscle and nerve functions, heart rhythm, immune system and bone formation. Simple, direct and automation-ready procedures for measuring magnesium concentration in biological samples are becoming popular in metabolomics research and drug discovery. The Magnesium Assay Kit is designed to measure magnesium directly in biological samples without any pretreatment. A calmagite dye in the kit forms a colored complex with magnesium. The intensity of the color, measured at 500 nm, is directly proportional to the magnesium concentration in the sample. The optimized formulation minimizes interference by potential substances.

The linear detection range of the kit is 0.1 mg/dL (41 µM) to 3 mg/dL (1.2 mM) Mg^{2+}. The kit is suitable for determination of magnesium in biological samples such as plasma, serum, and urine. The kit is also suitable for determination of magnesium in deproteinated samples such as milk and environmental samples such as water and soil.

Components

The kit is sufficient for 250 colorimetric assays in 96-well plates.

- Reagent A 25 mL
  Catalogue Number MAK507A
- Reagent B 25 mL
  Catalogue Number MAK507B
- EDTA Solution 1.5 mL
  Catalogue Number MAK507C
- Standard (10 mg/dL Mg^{2+}) 1 mL
  Catalogue Number MAK507D

Reagents and Equipment Required but Not Provided

- Pipetting devices and accessories (example, multichannel pipettor)
- Spectrophotometric multiwell plate reader.
- Clear bottom 96-well plates (example., Corning Costar). Cell culture or tissue culture treated plates are not recommended.
- 1.5 mL microcentrifuge tubes.
- Trichloroacetic Acid Solution (Catalogue number T0699)

Precautions and Disclaimer

For R&D use only. Not for drug, household, or other uses. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.

Storage/Stability

The kit is shipped at room temperature. Store all components at 4 °C.

Preparation Instructions

Briefly centrifuge small vials prior to opening. Equilibrate all components to room temperature prior to use.

Procedure

All Samples and Standards should be run in duplicate. This procedure is written for 96-well plates.

Note: The procedure may be used with cuvettes by scaling up the volumes 5x.

Sample Preparation

Serum and urine samples can be measured directly.

Note: EDTA and other Mg^{2+} chelators interfere with this assay. This assay cannot be applied to plasma samples obtained with EDTA.

Milk and Lipid/Protein Rich Samples:

1. Mix equal volumes of sample and 10% TCA.
2. Incubate at room temperature for 5 minutes.
3. Centrifuge for 2 minutes at 14,000 rpm.
4. Proceed using the supernatant for the assay.

Add 5 µL of each sample to separate wells in a clear, flat-bottom 96-well plate.

Standard Preparation

1. Dilute Standard to 2 mg/dL by mixing 40 µL of 10 mg/dL Standard with 160 µL purified water.
2. Transfer 5 µL of diluted standard into the 96-well plate.

Working Reagent

1. Prepare enough working reagent for each sample and standard.
2. Combine equal volumes of Reagent A and Reagent B.

Assay Reaction

1. Add 200 µL of Working Reagent to each Sample and Standard well. Tap plate to mix briefly and thoroughly.
2. Incubate for 2 minutes at room temperature.
3. Read optical density at 500 nm. This is the OD value for the samples and standard.
4. Add 10 µL EDTA solution to all wells and tap plate to mix thoroughly.
5. Incubate 2 minutes at room temperature.
6. Read optical density at 500 nm. This is the OD value for the blanks.

Results

Calculate the concentration of Mg^{2+} in the sample using the following equation:

$$Mg^{2+}\ \text{Conc.} = \frac{OD_{Sample} - OD_{Blank}}{OD_{Mg} - OD_{MgBlank}} \times 2\ (\text{mg/dL})$$

Where:

OD_{Sample} and OD_{Blank} are the optical density readings before and after the addition of EDTA.

OD_{Mg} and $OD_{MgBlank}$ are the optical density readings of the standard (2 mg/dL) before and after the addition of EDTA.

Conversions: 1 mg/dL Mg^{2+} equals 411 µM, 0.001% or 10 ppm.

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