BioChain®
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User's Manual and Instructions

Magnesium Assay Kit (Z5030032)

Quantitative Colorimetric Magnesium Determination at 500 nm

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. Magnesium deficiency may lead to nausea, fatigue, muscle contractions, hypocalcemia and hypokalemia.

Simple, direct and automation-ready procedures for measuring magnesium concentration in biological samples are becoming popular in Research and Drug Discovery. Biochain's 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 specifically 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.

KEY FEATURES

Sensitive and accurate. Use as little as 5 µL sample. Linear detection range 0.1 mg/dL (41µM) to 3 mg/dL (1.2 mM) Mg^{2+} in 96-well plate assay.
Simple and high-throughput. The procedure involves addition of two reagents and measuring OD_{500nm}. Can be readily automated as a high-throughput assay for thousands of samples per day.
Improved reagent stability and versatility. The optimized formulation has greatly enhanced reagent and signal stability. Cuvet or 96-well plate assay.
Low interference in biological samples. Assays can be directly performed in serum and urine samples.

APPLICATIONS

Direct Assays: Mg^{2+} in serum, urine and deproteinated samples (e.g. milk) etc.
Drug Discovery/Pharmacology: effects of drugs on Mg^{2+} metabolism.
Food and Beverages: Mg^{2+} determination.
Environment: Mg^{2+} determination in water and soil.

KIT CONTENTS (250 tests in 96-well plates)

Reagent A: 25 mL	**Reagent B: 25 mL**
EDTA Solution: 2 x 1.5 mL	**Standard: 1 mL 10 mg/dL Mg^{2+}**

Storage conditions. The kit is shipped at room temperature. Store Reagent and Standard at 4°C. Shelf life: 12 months after receipt.

Precautions: reagents are for research use only. Normal precautions for laboratory reagents should be exercised while using the reagents. Please refer to Material Safety Data Sheet for detailed information.

PROCEDURES

Reagent Preparation:

Prepare enough working reagent by combining equal volumes of Reagent A and Reagent B. Equilibrate to room temperature before use.

Procedure using 96-well plate:

1. Dilute Standard to 2 mg/dL by mixing 40 µL 10 mg/dL Standard with 160 µL distilled water. Transfer 5 µL diluted standard and samples in duplicate to wells of a clear bottom plate. Diluted standard can be stored at 4°C for future use.
2. Add 200 µL working reagent and tap plate to mix *thoroughly*.
3. Incubate 2 min at room temperature and read optical density at 500 nm (OD for *sample* and *standard*).
4. Add 10 µL EDTA Solution to all sample wells and tap plate to mix *thoroughly*. Incubate 2 min and read OD at 500nm (OD for *blanks*).

Procedure using cuvette:

1. Set up test tubes and transfer 25 µL diluted Standard and samples to appropriately labeled tubes.
2. Add 1000 µL working reagent and vortex to mix. Incubate 2 min. Transfer to cuvet and read OD_{500nm}. Add 50 µL EDTA solution, mix well, incubate 2 min and read $OD_{500\ nm}$.

CALCULATION

Magnesium concentration of the sample is calculated as

$$= \frac{OD_{SAMPLE} - OD_{BLANK}}{OD_{MG} - OD_{MGBlank}} \quad X\ 2\ (mg/dL)$$

OD_{SAMPLE} and OD_{BLANK} are OD_{500nm} values of sample before and after the addition of EDTA. OD_{MG} and $OD_{MGBLANK}$ are OD_{500nm} values 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.

MATERIALS REQUIRED, BUT NOT PROVIDED

Pipeting devices and accessories (e.g. 5 µL).

Procedure using 96-well plate:

Clear bottom 96-well plates (e.g. Corning Costar) and plate reader.

Procedure using cuvette:

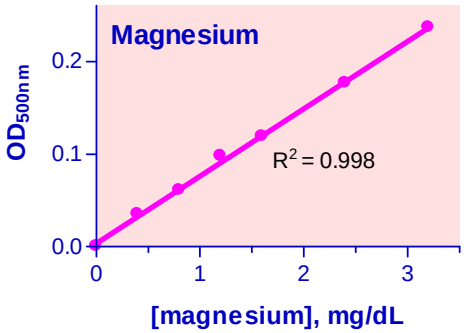
Cuvets and spectrophotometer for measuring OD_{500nm}.

GENERAL CONSIDERATIONS

1. EDTA and other Mg^{2+} chelators interfere with this assay. This assay can not be applied to plasma samples obtained with EDTA. 2. Sample pretreatment: for milk and other lipid/protein-rich samples, mix equal volumes of sample and 10% trichloroacetic acid (Sigma Cat# T6399). Incubate 5 min at room temperature and pellet precipitates for 2 min at 14,000 rpm in a table centrifuge. Assay the supernatant (dilution factor = 2) using the above procedure.

EXAMPLES

Samples were assayed in duplicate using the 96-well plate protocol. The Mg^{2+} values (mg/dL) were 1.64 ± 0.04 (rat serum), 1.77 ± 0.02 (human serum), 2.41 ± 0.5 (goat serum), 2.80 ± 0.14 (Invitrogen fetal bovine serum).



Calibration curve in 96-well plate

LITERATURE

[1]. Kim, T. et al (2006). Rapid production of milligram quantities of proteins in a batch cell-free protein synthesis system. J. Biotechnol. 124(2): 373-383.

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