

## QuantiChrom™ Magnesium Assay Kit (DIMG-250)

### 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. BioAssay Systems' 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  $\mu$ L sample. Linear detection range 0.1 mg/dL (41 $\mu$ M) to 3 mg/dL (1.2 mM) Mg<sup>2+</sup> in 96-well plate assay.

**Simple and high-throughput.** The procedure involves addition of two reagents and measuring OD<sub>500nm</sub>. 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<sup>2+</sup> in serum, urine and deproteinated samples (e.g. milk) etc.

**Drug Discovery/Pharmacology:** effects of drugs on Mg<sup>2+</sup> metabolism.

**Food and Beverages:** Mg<sup>2+</sup> determination.

**Environment:** Mg<sup>2+</sup> 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<sup>2+</sup>

**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  $\mu$ L 10 mg/dL Standard with 160  $\mu$ L distilled water. Transfer 5  $\mu$ L 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  $\mu$ L 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  $\mu$ L 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  $\mu$ L diluted Standard and samples to appropriately labeled tubes.
2. Add 1000  $\mu$ L working reagent and vortex to mix. Incubate 2 min. Transfer to cuvet and read OD<sub>500nm</sub>. Add 50  $\mu$ L EDTA solution, mix

well, incubate 2 min and read OD<sub>500 nm</sub>.

#### CALCULATION

Magnesium concentration of the sample is calculated as

$$= \frac{OD_{\text{SAMPLE}} - OD_{\text{BLANK}}}{OD_{\text{MG}} - OD_{\text{MGBLANK}}} \times 2 \text{ (mg/dL)}$$

OD<sub>SAMPLE</sub> and OD<sub>BLANK</sub> are OD<sub>500nm</sub> values of sample before and after the addition of EDTA. OD<sub>MG</sub> and OD<sub>MGBLANK</sub> are OD<sub>500nm</sub> values of the standard (2 mg/dL) before and after the addition of EDTA.

**Conversions:** 1 mg/dL Mg<sup>2+</sup> equals 411  $\mu$ M, 0.001% or 10 ppm.

#### MATERIALS REQUIRED, BUT NOT PROVIDED

Pipeting devices and accessories (e.g. 5  $\mu$ L).

##### Procedure using 96-well plate:

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

##### Procedure using cuvette:

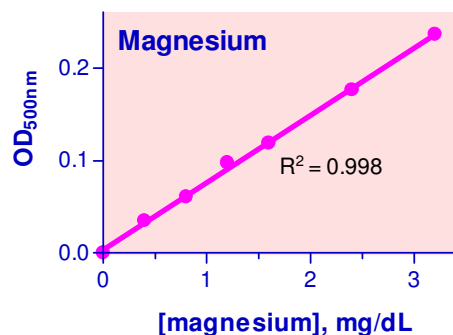
Cuvets and spectrophotometer for measuring OD<sub>500nm</sub>.

#### GENERAL CONSIDERATIONS

1. EDTA and other Mg<sup>2+</sup> 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<sup>2+</sup> values (mg/dL) were 1.64  $\pm$  0.04 (rat serum), 1.77  $\pm$  0.02 (human serum), 2.41  $\pm$  0.5 (goat serum), 2.80  $\pm$  0.14 (Invitrogen fetal bovine serum).



Standard Curve in 96-well plate assay

#### PUBLICATIONS

1. Breiderhoff, T et al (2012). Deletion of claudin-10 (Cldn10) in the thick ascending limb impairs paracellular sodium permeability and leads to hypermagnesemia and nephrocalcinosis. Proceedings of the National Academy of Sciences 109(35): 14241-14246.
2. Stippler, M et al (2007). Serum and cerebrospinal fluid magnesium in severe traumatic brain injury outcome. J. Neurotrauma. 24(8): 1347-1354.
3. Elizondo, MR et al (2010). trpm7 regulation of in vivo cation homeostasis and kidney function involves stanniocalcin 1 and fgf23. Endocrinology 151(12):5700-5709