



Magnesium Assay Kit

Catalog Number KA0813

100 assays

Version: 02

Intended for research use only

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Introduction

Background

Magnesium is the 11th most abundant element by mass in the human body. Mg^{+2} is essential to all living cells where it plays an important role in facilitating the processing of biological polyphosphates like ATP, DNA, RNA and enzyme functions. Mg^{+2} is the metallic ion at the center of chlorophyll, and a common additive to fertilizers. Mg^{+2} compounds are used as laxatives, antacids, and used to stabilize abnormal nerve excitation and blood vessel spasm i.e., eclampsia. The Magnesium Assay Kit provides a simple sensitive means of quantitating magnesium in a variety of biological samples. The kit takes advantage of the specific requirement of glycerol kinase for Mg^{+2} . An enzyme linked reaction leads to formation of an intensely colored ($\lambda_{max} = 450\text{ nm}$) product whose formation is proportional to Mg^{+2} concentration. The linear range of the assay is 2-15 nmoles with detection sensitivity~ 40 μM .

General Information

Materials Supplied

List of component

Component	Amount
Magnesium Assay Buffer	25 mL
Magnesium Developer: lyophilized.	1 vial
Magnesium Enzyme Mix: lyophilized.	1 vial
Magnesium Standard (150 nmol/ μ L)	0.1 mL

Storage Instruction

Store kit at -20°C, protect from light. Warm buffer to room temperature before use. Briefly centrifuge all small vials prior to opening.

Assay Protocol

Reagent Preparation

- Developer: Dissolve with 1.1 mL dH₂O. Stable for two months at 4°C.
- Magnesium Enzyme Mix: Dissolve in 550 µL Assay Buffer. Aliquot and store at -20°C. Use within two months.
- Magnesium Standard: Ready to use as supplied. 150 nmol/µL of Mg²⁺ Standard stock solution. Store at -20°C. Mix before each use.

Assay Procedure

1. Standard Curve Preparations:
Dilute the standard to 1.5 nmol/µL by adding 10 µL of the 150 nmol/µL Magnesium Standard to 990 µL of distilled water, mix well. Add 0, 2, 4, 6, 8, 10 µL into a series of wells. Adjust volume to 50 µL/well with distilled water to generate 0, 3, 6, 9, 12, 15 nmol/well of Magnesium Standard.
2. Sample Preparation:
Tissue or cells can be extracted with 4 volume of Magnesium Assay Buffer, spin 16000g for 10 min to get clear extract. Add 1-50 µL of liquid sample into 96 well plate, bring total volume to 50 µL with water. Normal serum contains Mg²⁺ 0.7-1.05 mM (1.65-2.55 mg/dL), use 5 µL serum for testing. Urine should be diluted 10X. For unknown samples, we suggest testing different amount of samples to ensure OD is in the linear range.
3. Magnesium Reaction Mix: Mix enough reagent for the number of samples and standards to be performed:
For each well, prepare a total 50 µL Reaction Mix containing:
 - 35 µL Magnesium Assay Buffer
 - 10 µL Developer
 - 5 µL Magnesium Enzyme Mix
4. Add 50 µL of the Reaction Mix to each well containing the Magnesium Standard and test samples. For best results, use a multichannel pipettor to initiate reaction in all samples at the same time. Mix well.
5. Incubate at 37°C for 10 min. Read the plate OD_{450 nm} to get A₀ for each standard or sample.
Notes:
 - ✓ Since enzyme kinetics are sensitive to temperature variation, the reaction rate will increase as the temperature rises. The reaction takes ~ 10 minutes to reach a linear reaction rate.
 - ✓ NAD(P)H etc. in samples may generate background, the 10 min waiting time can correct these nonspecific background.
 - ✓ Mn²⁺, Zn²⁺, Ni²⁺, Fe²⁺, Cu²⁺, Co²⁺, Ca²⁺ do not interfere with the assay.
6. Incubate the reaction for additional 10-30 min, read the OD again to get reading A. We recommend monitor the reaction kinetics to ensure the readings are in linear range when read the plate for the additional 10-30 minutes. All readings should not exceed 1.5 OD

Data Analysis

Calculation of Results

Subtract A_0 from standard and sample readings to get $\Delta OD = A - A_0$. Plot Magnesium standard curve. Apply sample ΔOD to the standard curve to get Mg^{2+} amount B (nmol) in the reaction well. Mg^{2+} concentration:

$$C = B / V \text{ (nmol/mL or } \mu\text{M)}$$

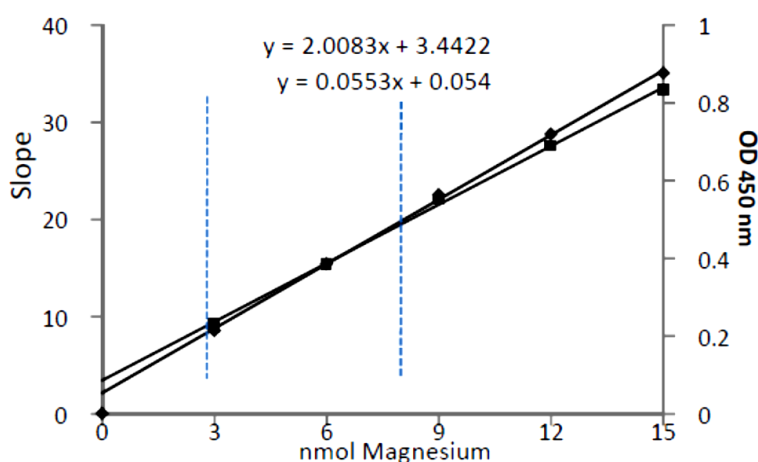
Where:

B is Mg^{2+} amount in the reaction well (in nmol).

V is the sample volume added into the reaction well (in mL).

Magnesium molecular weight: 24.3 g/mol, 1 mM = 2.43 mg/dL.

The assay may also be calculated by monitoring reaction slopes in the standards and samples reaction.



Magnesium standard curve: Assay is performed according to kit protocol. Vertical dotted lines indicate the lower and upper limits of normal serum Mg^{2+} concentrations.