

Magnesium Colorimetric Assay Kit

(Catalog #K385-100; 100 assays; Store at -20°C)

I. Introduction:

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 BioVision 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 ($\lambda_{max} = 450nm$) product whose formation is proportional to Mg^{+2} concentration. The linear range of the assay is 2-15 nmoles with detection sensitivity~ 40 μM .

II. Kit Contents:

Components	K385-100	Cap Code	Part Number
Magnesium Assay Buffer	25 ml	WM	K385-100-1
Magnesium Developer	lyophilized	Red	K385-100-2
Magnesium Enzyme Mix	lyophilized	Green	K385-100-3
Magnesium Standard (150 nmol/ μ l)	0.1 ml	Yellow	K385-100-4

III. Storage and Handling:

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

Reagent Preparation:

Developer: Dissolve with 1.1 ml dH₂O. Stable for two months at 4°C.

Magnesium Enzyme Mix: Dissolve in 550 μ l Assay Buffer. Aliquot and store at -20°C. Use within two months.

Magnesium Standard: Ready to use as supplied. 150 nmol/ μ l of Mg^{+2} Standard stock solution. Store at -20°C. Mix before each use.

IV. Magnesium Assay Protocol:

1. Standard Curve Preparations:

Dilute the standard to 1.5 nmol/ μ l by adding 10 μ l of the 150 nmol/ μ l Magnesium Standard to 990 μ l of distilled water, mix well. Add 0, 2, 4, 6, 8, 10 μ l into a series of wells. Adjust volume to 50 μ l/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 μ l of liquid sample into 96 well plate, bring total volume to 50 μ l with water.

Normal serum contains Mg^{2+} 0.7-1.05 mM (1.65-2.55 mg/dL), use 5 μ l 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 μ l Reaction Mix containing:

- 35 μ l Magnesium Assay Buffer
- 10 μ l Developer
- 5 μ l Magnesium Enzyme Mix

4. Add 50 μ l 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_{450nm} to get A₀ for each standard or sample.

Notes:

- 1) 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.
- 2) NAD(P)H etc. in samples may generate background, the 10 min waiting time can correct these nonspecific background.
- 3) Mn^{2+} , Zn^{2+} , Ni^{2+} , Fe^{2+} , Cu^{2+} , Co^{2+} , Ca^{2+} 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.
7. **Calculation:** Subtract A₀ 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:

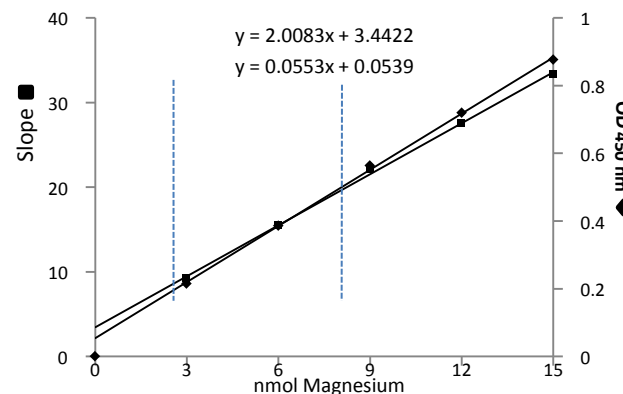
$$C = B/V \text{ (nmol/ml or } \mu M)$$

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.

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