RayBio® Magnesium Assay Kit

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RayBio® Magnesium Assay Kit Protocol

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RayBiotech, Inc.

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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 RayBiotech 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 (λ_{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

Store Kit at -20°C

Components	Size	Cap Code	Part Number
Magnesium Assay Buffer	25 ml	WM	Item A
Magnesium Developer	Lyophilized	Red	Item B
Magnesium Enzyme Mix	Lyophilized	Green	Item C
Magnesium Standard (150 nmol/µl)	0.1 ml	Yellow	Item D

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.

IV. REAGENT PREPARATION

Developer: Dissolve with 1.1 ml dH_2O. 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 Mg^{+2} Standard stock solution. Store at –20°C. Mix before each use.

VII. MAGNESIUM ASSAY PROTOCOL

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 Mg^{2+} 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 mim. Read the plate $OD450_{nm}$ to get A_0 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.
- Mn^{2+}, Zn^{2+}, Ni^{2+}, Fe^{2+}, Cu^{2+}, Co^{2+}, Ca^{2+} do not interfere with the assay.

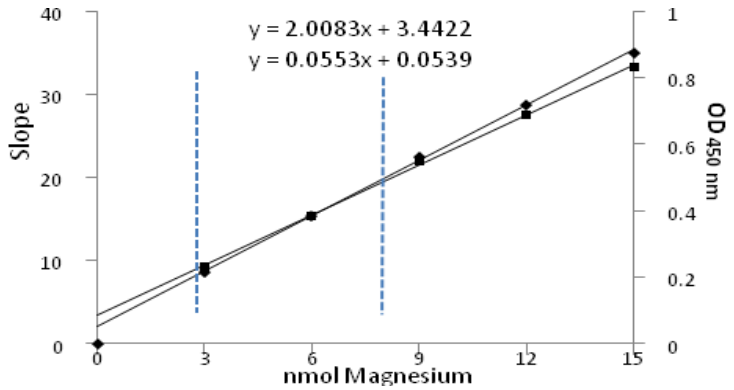
6) Incubate the reaction for additional 10-30 min, read the OD again to get reading A. We recommend monitor the reaction kinetics to ensure the readings are in linear range when read the plate for the additional 10-30 minutes. All readings should not exceed 1.5 OD.

7) Calculation: Subtract 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:

$$\mathbf{C} = \mathbf{B}/\mathbf{V} \qquad \text{(nmol/ml or µM)}$$

Where: **B** is Mg^{2+} amount in reaction well (in nmol).
V is the sample volume added into the reaction well (in ml).
Magnesium molecular weight: 24.3 g/mol, 1mM=2.43 mg/dL.

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



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.

This product is for research use only.