ab102506

Magnesium Detection Kit

Instructions for Use

For the rapid, sensitive and accurate detection of Magnesium in various samples

This product is for research use only and is not intended for diagnostic use.

PLEASE NOTE: With the acquisition of BioVision by Abcam, we have made some changes to component names and packaging to better align with our global standards as we work towards environmental-friendly and efficient growth. You are receiving the same high-quality products as always, with no changes to specifications or protocols.

Version 5a, Last Updated 15 August 2023

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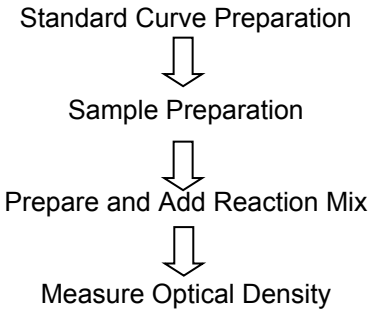
1. Overview

Magnesium (Mg^{2+}) is the 11th most abundant element by mass in the human body. Magnesium 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.

Magnesium is the metallic ion at the center of chlorophyll, and a common additive to fertilizers. Magnesium compounds are used as laxatives, antacids, and to stabilize abnormal nerve excitation and blood vessel spasm i.e., eclampsia.

Abcam's 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 Magnesium. An enzyme linked reaction leads to formation of an intensely colored (λ_{max} = 450nm) product whose formation is proportional to Magnesium concentration. The linear range of the assay is 2-15 nmoles with detection sensitivity ~40 µM.

2. Protocol Summary



3. Materials Supplied

Item	Quantity
Magnesium Assay Buffer	25 mL
Magnesium Enzyme Mix/Magnesium Enzyme Mix (Lyophilized)	1 vial
Developer Solution III/Magnesium Developer (Lyophilized)	1 vial
Magnesium Standard/Magnesium Standard (150 nmol/µL)	100 µL

4. Storage and Stability

Upon arrival, store the kit at -20°C and protected from light.

Briefly centrifuge all small vials prior to opening.

Read the entire protocol before performing the assay.

MAGNESIUM ASSAY BUFFER: warm buffer to room temperature before us.

DEVELOPER SOLUTION III/MAGNESIUM DEVELOPER: dissolve in 1.1 ml dH_2O. Reconstituted developer is 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 a 150 nmol/µl Mg^{2+} Standard Stock solution. Store at -20°C. Mix well before each use.

5. Materials Required, Not Supplied

- Distilled water or MilliQ
- Microcentrifuge
- Pipettes and pipette tips
- Colorimetric microplate reader
- 96-well plate
- Orbital shaker

6. Assay Protocol

1. Standard Curve Preparation:

Prepare a 1.5 nmol/µl Mg^{2+} Standard by diluting 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:

a. **For tissue or cell samples:** Tissue or cells can be extracted with 4x volumes of Magnesium Assay Buffer, spin 16000 x g 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.

b. **For serum samples:** Normal serum contains Mg^{2+} 0.7-1.05 mM (1.65-2.55 mg/dL); use 5 µl of serum for testing. Bring total volume to 50 µl with dH_2O.

c. **For urine samples:** Urine should be diluted 10X. Bring total volume to 50 µl with dH_2O.

d. **Other liquid samples (cell culture media and other biological fluids):** liquid samples can be assayed directly or after bringing total volume to 50 µl with dH_2O. You might want to test different

sample volumes to find the optimal that will give you a reading within the linear range of the standard curve.

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:

Magnesium Assay Buffer	35 µl
Developer	10 µl
Magnesium Enzyme Mix	5 µl

Add 50 µl of the Reaction Mix to each well containing the Magnesium Standard and test samples. Incubate at 37°C for 10 min.

Note: *For best results, use a multichannel pipette to initiate reaction in all samples at the same time. Mix well.*

4. Measurement:

BEFORE YOU START MEASURING:

a) Since enzyme kinetics are sensitive to temperature variation, the reaction rate will increase as the temperature rises. The reaction takes ~10 min to reach a linear reaction rate.

b) NAD(P)H etc. in samples may generate background, the 10 min waiting time can correct these nonspecific background.

c) Mn^{2+}, Zn^{2+}, Ni^{2+}, Fe^{2+}, Cu^{2+}, Co^{2+}, Ca^{2+} do not interfere with the assay.

After the initial 10 min-incubation, read the plate OD_{450nm} to get A_0 for each standard or sample.

Incubate the reaction for additional 10 – 30 minutes, and read the OD again to get reading A. We recommend monitor the reaction kinetics to ensure the readings are in linear range when reading the plate for the additional 10 – 30 minutes. All readings should be at OD_{450nm} < 1.5 OD.

7. Data Analysis

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 Magnesium amount B (nmol) in the reaction well.

Calculate Magnesium concentration:

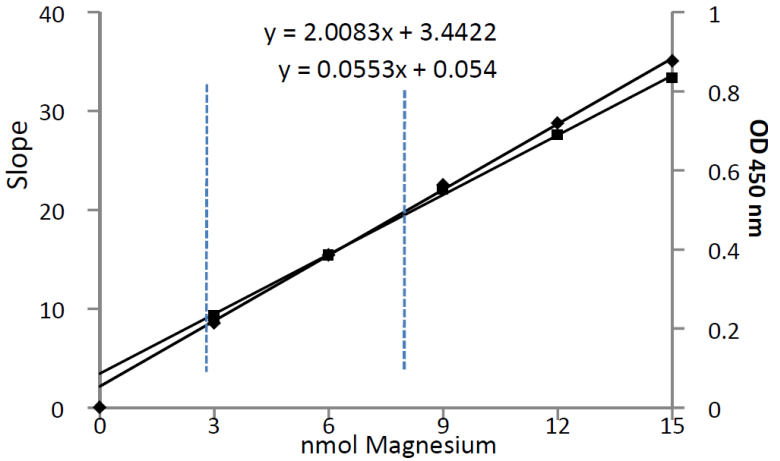
Concentration = B / V (nmol/ml or μM)

Where:

B is Magnesium 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.



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

8. Troubleshooting

Problem	Reason	Solution
Assay not working	Assay buffer at wrong temperature	Assay buffer must not be chilled - needs to be at RT
	Protocol step missed	Re-read and follow the protocol exactly
	Plate read at incorrect wavelength	Ensure you are using appropriate reader and filter settings (refer to datasheet)
	Unsuitable microtiter plate for assay	Fluorescence: Black plates (clear bottoms); Luminescence: White plates; Colorimetry: Clear plates. If critical, datasheet will indicate whether to use flat- or U-shaped wells
Unexpected results	Measured at wrong wavelength	Use appropriate reader and filter settings described in datasheet
	Samples contain impeding substances	Troubleshoot and also consider deproteinizing samples
	Unsuitable sample type	Use recommended samples types as listed on the datasheet
	Sample readings are outside linear range	Concentrate/ dilute samples to be in linear range

Samples with inconsistent readings	Unsuitable sample type	Refer to datasheet for details about incompatible samples
	Samples prepared in the wrong buffer	Use the assay buffer provided (or refer to datasheet for instructions)
	Samples not deproteinized (if indicated on datasheet)	Use the ***10kDa spin column (ab93349)***
	Cell/ tissue samples not sufficiently homogenized	Increase sonication time/ number of strokes with the Dounce homogenizer
	Too many freeze-thaw cycles	Aliquot samples to reduce the number of freeze-thaw cycles
	Samples contain impeding substances	Troubleshoot and also consider deproteinizing samples
	Samples are too old or incorrectly stored	Use freshly made samples and store at recommended temperature until use
Lower/ Higher readings in samples and standards	Not fully thawed kit components	Wait for components to thaw completely and gently mix prior use
	Out-of-date kit or incorrectly stored reagents	Always check expiry date and store kit components as recommended on the datasheet
	Reagents sitting for extended periods on ice	Try to prepare a fresh reaction mix prior to each use
	Incorrect incubation time/ temperature	Refer to datasheet for recommended incubation time and/ or temperature
	Incorrect amounts used	Check pipette is calibrated correctly (always use smallest volume pipette that can pipette entire volume)

Problem	Reason	Solution
Standard curve is not linear	Not fully thawed kit components	Wait for components to thaw completely and gently mix prior use
	Pipetting errors when setting up the standard curve	Try not to pipette too small volumes
	Incorrect pipetting when preparing the reaction mix	Always prepare a master mix
	Air bubbles in wells	Air bubbles will interfere with readings; try to avoid producing air bubbles and always remove bubbles prior to reading plates
	Concentration of standard stock incorrect	Recheck datasheet for recommended concentrations of standard stocks
	Errors in standard curve calculations	Refer to datasheet and re-check the calculations
	Use of other reagents than those provided with the kit	Use fresh components from the same kit

For further technical questions please do not hesitate to contact us by email (technical@abcam.com) or phone (select "*contact us*" on www.abcam.com for the phone number for your region).

Technical Support

For all technical or commercial enquiries please go to:

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