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.

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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 ofchlorophyll, 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 quantitimating 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 ($\lambda_{\text{max}} = 450$nm) product whose formation is proportional to Magnesium concentration. The linear range of the assay is 2-15 nmoles with detection sensitivity $\sim 40$ µM.
2. Protocol Summary

Standard Curve Preparation

↓

Sample Preparation

↓

Prepare and Add Reaction Mix

↓

Measure Optical Density
3. Materials Supplied

<table>
<thead>
<tr>
<th>Item</th>
<th>Quantity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Magnesium Assay Buffer</td>
<td>25 mL</td>
</tr>
<tr>
<td>Magnesium Enzyme Mix (Lyophilized)</td>
<td>1 vial</td>
</tr>
<tr>
<td>Magnesium Developer (Lyophilized)</td>
<td>1 vial</td>
</tr>
<tr>
<td>Magnesium Standard (150 nmol/µL)</td>
<td>100 µL</td>
</tr>
</tbody>
</table>

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.

MAGNESIUM DEVELOPER: dissolve in 1.1 ml dH₂O. 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\(_2\)O.

   c. For urine samples: Urine should be diluted 10X. Bring total volume to 50 µl with dH\(_2\)O.

   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\(_2\)O. 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$_{450\text{nm}}$ 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$_{450\text{nm}} < 1.5$ 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 $\Delta OD$ to the standard curve to get Magnesium amount $B$ (nmol) in the reaction well.

Calculate Magnesium concentration:

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

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

<table>
<thead>
<tr>
<th>Problem</th>
<th>Reason</th>
<th>Solution</th>
</tr>
</thead>
<tbody>
<tr>
<td>Assay not working</td>
<td>Assay buffer at wrong temperature</td>
<td>Assay buffer must not be chilled - needs to be at RT</td>
</tr>
<tr>
<td>Protocol step missed</td>
<td></td>
<td>Re-read and follow the protocol exactly</td>
</tr>
<tr>
<td>Plate read at incorrect wavelength</td>
<td></td>
<td>Ensure you are using appropriate reader and filter settings (refer to datasheet)</td>
</tr>
<tr>
<td>Unsuitable microtiter plate for assay</td>
<td></td>
<td>Fluorescence: Black plates (clear bottoms); Luminescence: White plates; Colorimetry: Clear plates. If critical, datasheet will indicate whether to use flat- or U-shaped wells</td>
</tr>
<tr>
<td>Unexpected results</td>
<td>Measured at wrong wavelength</td>
<td>Use appropriate reader and filter settings described in datasheet</td>
</tr>
<tr>
<td>Samples contain impeding substances</td>
<td></td>
<td>Troubleshoot and also consider deproteinizing samples</td>
</tr>
<tr>
<td>Unsuitable sample type</td>
<td></td>
<td>Use recommended samples types as listed on the datasheet</td>
</tr>
<tr>
<td>Sample readings are outside linear range</td>
<td></td>
<td>Concentrate/ dilute samples to be in linear range</td>
</tr>
<tr>
<td>Samples with inconsistent readings</td>
<td>Unsuitable sample type</td>
<td>Refer to datasheet for details about incompatible samples</td>
</tr>
<tr>
<td>------------------------------------</td>
<td>------------------------</td>
<td>-----------------------------------------------------------</td>
</tr>
<tr>
<td>Samples prepared in the wrong buffer</td>
<td>Use the assay buffer provided (or refer to datasheet for instructions)</td>
<td></td>
</tr>
<tr>
<td>Samples not deproteinized (if indicated on datasheet)</td>
<td>Use the <strong>10kDa spin column (ab93349)</strong></td>
<td></td>
</tr>
<tr>
<td>Cell/tissue samples not sufficiently homogenized</td>
<td>Increase sonication time/number of strokes with the Dounce homogenizer</td>
<td></td>
</tr>
<tr>
<td>Too many freeze-thaw cycles</td>
<td>Aliquot samples to reduce the number of freeze-thaw cycles</td>
<td></td>
</tr>
<tr>
<td>Samples contain impeding substances</td>
<td>Troubleshoot and also consider deproteinizing samples</td>
<td></td>
</tr>
<tr>
<td>Samples are too old or incorrectly stored</td>
<td>Use freshly made samples and store at recommended temperature until use</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Lower/ Higher readings in samples and standards</th>
<th>Not fully thawed kit components</th>
<th>Wait for components to thaw completely and gently mix prior use</th>
</tr>
</thead>
<tbody>
<tr>
<td>Out-of-date kit or incorrectly stored reagents</td>
<td>Always check expiry date and store kit components as recommended on the datasheet</td>
<td></td>
</tr>
<tr>
<td>Reagents sitting for extended periods on ice</td>
<td>Try to prepare a fresh reaction mix prior to each use</td>
<td></td>
</tr>
<tr>
<td>Incorrect incubation time/temperature</td>
<td>Refer to datasheet for recommended incubation time and/or temperature</td>
<td></td>
</tr>
<tr>
<td>Incorrect amounts used</td>
<td>Check pipette is calibrated correctly (always use smallest volume pipette that can pipette entire volume)</td>
<td></td>
</tr>
<tr>
<td>Problem</td>
<td>Reason</td>
<td>Solution</td>
</tr>
<tr>
<td>---------------------------------</td>
<td>------------------------------------------------------------------------</td>
<td>--------------------------------------------------------------------------</td>
</tr>
<tr>
<td>Standard curve is not linear</td>
<td>Not fully thawed kit components</td>
<td>Wait for components to thaw completely and gently mix prior use</td>
</tr>
<tr>
<td></td>
<td>Pipetting errors when setting up the standard curve</td>
<td>Try not to pipette too small volumes</td>
</tr>
<tr>
<td></td>
<td>Incorrect pipetting when preparing the reaction mix</td>
<td>Always prepare a master mix</td>
</tr>
<tr>
<td></td>
<td>Air bubbles in wells</td>
<td>Air bubbles will interfere with readings; try to avoid producing air bubbles and always remove bubbles prior to reading plates</td>
</tr>
<tr>
<td></td>
<td>Concentration of standard stock incorrect</td>
<td>Recheck datasheet for recommended concentrations of standard stocks</td>
</tr>
<tr>
<td></td>
<td>Errors in standard curve calculations</td>
<td>Refer to datasheet and re-check the calculations</td>
</tr>
<tr>
<td></td>
<td>Use of other reagents than those provided with the kit</td>
<td>Use fresh components from the same kit</td>
</tr>
</tbody>
</table>

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).
UK, EU and ROW
Email: technical@abcam.com | Tel: +44-(0)1223-696000

Austria
Email: wissenschaftlicherdienst@abcam.com | Tel: 019-288-259

France
Email: supportscientifique@abcam.com | Tel: 01-46-94-62-96

Germany
Email: wissenschaftlicherdienst@abcam.com | Tel: 030-896-779-154

Spain
Email: soportecientifico@abcam.com | Tel: 911-146-554

Switzerland
Email: technical@abcam.com
Tel (Deutsch): 0435-016-424 | Tel (Français): 0615-000-530

US and Latin America
Email: us.technical@abcam.com | Tel: 888-77-ABCAM (22226)

Canada
Email: ca.technical@abcam.com | Tel: 877-749-8807

China and Asia Pacific
Email: hk.technical@abcam.com | Tel: 400 921 0189 / +86 21 2070 0500

Japan
Email: technical@abcam.co.jp | Tel: +81-(0)3-6231-0940

www.abcam.com | www.abcam.cn | www.abcam.co.jp