**Product Information**

**Magnesium Assay Kit**

Catalog Number MAK026  
Storage Temperature –20 °C

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**TECHNICAL BULLETIN**

**Product Description**

Magnesium, one of the most abundant trace metals in living organisms, contributes to a variety of biological functions including ATP and nucleic acid processing, energy metabolism, and enzymatic function. Magnesium forms complexes with multiple molecules such as phospholipids and ATP. Low serum levels of magnesium have been associated with metabolic syndrome, diabetes mellitus type 2, and hypertension.

The Magnesium Assay kit provides a simple and direct procedure for measuring magnesium in a variety of samples. The magnesium concentration is determined by a coupled enzyme assay that takes advantage of the specific requirement of glycerol kinase for Mg²⁺, resulting in a colorimetric (450 nm) product proportional to the magnesium present. This assay gives a linear range of 3–15 nmoles of magnesium and exhibits no detectable interference with Fe²⁺, Cu²⁺, Ni²⁺, Zn²⁺, Co²⁺, Ca²⁺, and Mn²⁺.

**Components**

The kit is sufficient for 100 assays in 96 well plates.

- **Magnesium Assay Buffer**  
  Catalog Number MAK026A  
  25 mL

- **Magnesium Developer**  
  Catalog Number MAK026B  
  1 vl

- **Magnesium Enzyme Mix**  
  Catalog Number MAK026D  
  1 vl

- **Magnesium Standard, 150 nmole/µL**  
  Catalog Number MAK026E  
  0.1 mL

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**Reagents and Equipment Required but Not Provided.**

- 96 well flat-bottom plate – It is recommended to use clear plates for colorimetric assays.
- Spectrophotometric multiwell plate reader

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**Precautions and Disclaimer**

This product is for R&D use only, not for drug, household, or other uses. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.

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**Preparation Instructions**

Briefly centrifuge vials before opening. Use ultrapure water for the preparation of reagents. To maintain reagent integrity, avoid repeated freeze/thaw cycles.

- **Magnesium Assay Buffer** – Allow buffer to come to room temperature before use.

- **Magnesium Developer** – Reconstitute vial with 1.1 mL of water. Mix well by pipetting (don’t vortex), then aliquot and store, protected from light, at 2–8 °C. Use within 2 months of reconstitution.

- **Magnesium Enzyme Mix** – Reconstitute with 550 µL of Magnesium Assay Buffer. Mix well by pipetting, then aliquot and store, protected from light, at –20 °C. Use within 2 months of reconstitution.

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**Storage/Stability**

The kit is shipped on wet ice and storage at –20 °C, protected from light, is recommended.
Procedure
All samples and standards should be run in duplicate.

Magnesium Standards for Colorimetric Detection
Dilute 10 μL of the 150 nmole/μL Magnesium Standard with 990 μl of water to prepare a 1.5 nmole/μL standard solution. Add 0, 2, 4, 6, 8, and 10 μL of the 1.5 nmole/μL standard solution into a 96 well plate, generating 0 (blank), 3, 6, 9, 12, and 15 nmole/well standards. Add water to each well to bring the volume to 50 μL.

Sample Preparation
Tissue (20 mg) or cells (2 × 10^6) can be homogenized in 4 volumes of ice-cold Magnesium Assay Buffer. Centrifuge the samples at 13,000 × g for 10 minutes to remove insoluble material.

Serum (1–5 μL) can be added directly to well. Urine should be diluted 10-fold before adding to well.

1–50 μL of liquid samples can be added directly to wells.

Note: For unknown samples, it is suggested to test several sample dilutions to ensure the readings are within the linear range of the standard curve.

Bring samples to a final volume of 50 μL with water.

Assay Reaction
1. Set up the Master Reaction Mix according to the scheme in Table 1. 50 μL of the Master Reaction Mix is required for each reaction (well).

Table 1. Master Reaction Mix

<table>
<thead>
<tr>
<th>Reagent</th>
<th>Volume</th>
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<tr>
<td>Magnesium Assay Buffer</td>
<td>35 μL</td>
</tr>
<tr>
<td>Developer</td>
<td>10 μL</td>
</tr>
<tr>
<td>Magnesium Enzyme Mix</td>
<td>5 μL</td>
</tr>
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</table>

2. Add 50 μL of the Master Reaction Mix to each of the wells. Mix well using a horizontal shaker or by pipetting, and incubate the reaction for 10 minutes at 37 °C. Cover the plate and protect from light during the incubation.

3. Measure the absorbance at 450 nm at the initial time (A_{450})_{initial}.
   Note: It is essential (A_{450})_{initial} is in the linear range of the standard curve.

4. Continue to incubate the plate at 37 °C taking measurements (A_{450}) every 5 minutes until the highest A_{450} approaches 1.5 (A_{450})_{final}. Protect the plate from light during the incubation. No A_{450} readings should exceed 1.5.

Notes: The reaction takes ~10 minutes to reach a linear reaction rate.

NADPH in the samples may generate background. The 10 minute initial reading can be used to correct for nonspecific background.
**Results**

**Calculations**

Use the values obtained from the appropriate magnesium standards to plot a standard curve.

*Note:* A new standard curve must be set up each time the assay is run.

Subtract the $(A_{450})_{\text{initial}}$ value from the $(A_{450})_{\text{final}}$ value for each reading to obtain the corrected measurement. Using the corrected measurement, the amount of magnesium present in the samples may be determined from the standard curve.

Concentration of Magnesium

\[
\frac{S_a}{S_v} = C
\]

- $S_a$ = Amount of magnesium in unknown sample (nmole) from standard curve
- $S_v$ = Sample volume (μL) added to reaction well
- $C$ = Concentration of magnesium in sample

Magnesium atomic weight: 24.3 g/mole

Sample Calculation

Amount of magnesium $(S_a) = 5.84$ nmole (from standard curve)
Sample volume $(S_v) = 50$ μL

Concentration of magnesium in sample

\[
5.84 \text{ nmole/50 } \mu\text{L} = 0.1168 \text{ nmole/μL}
\]

\[
0.1168 \text{ nmole/μL} \times 24.3 \text{ ng/nmole} = 2.84 \text{ ng/μL}
\]
## Troubleshooting Guide

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<th>Possible Cause</th>
<th>Suggested Solution</th>
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<td>Refer and follow Technical Bulletin precisely</td>
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<td>Plate reader at incorrect wavelength</td>
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<td>Type of 96 well plate used</td>
<td>For colorimetric assays, use clear plates</td>
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<tr>
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<td>Samples prepared in different buffer</td>
<td>Use the Assay Buffer provided or refer to Technical Bulletin for instructions</td>
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<tr>
<td></td>
<td>Cell/Tissue culture samples were incompletely homogenized</td>
<td>Repeat the sample homogenization, increasing the length and extent of homogenization step.</td>
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<td>Samples used after multiple freeze-thaw cycles</td>
<td>Aliquot and freeze samples if needed to use multiple times</td>
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<td>Presence of interfering substance in the sample</td>
<td>If possible, dilute sample further</td>
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<td>Thaw all components completely and mix gently before use</td>
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<td>Use of expired kit or improperly stored reagents</td>
<td>Check the expiration date and store the components appropriately</td>
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<tr>
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<td>Allowing the reagents to sit for extended times on ice</td>
<td>Prepare fresh Master Reaction Mix before each use</td>
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<td>Refer to Technical Bulletin and verify correct incubation times and temperatures</td>
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<td>Incorrect volumes used</td>
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<tr>
<td>Non-linear standard curve</td>
<td>Use of partially thawed components</td>
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<td></td>
<td>Pipetting errors in preparation of standards</td>
<td>Avoid pipetting small volumes</td>
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<tr>
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<td>Pipetting errors in the Reaction Mix</td>
<td>Prepare a Master Reaction Mix whenever possible</td>
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<td>Air bubbles formed in well</td>
<td>Pipette gently against the wall of the plate well</td>
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<td>Standard stock is at incorrect concentration</td>
<td>Refer to the standard dilution instructions in the Technical Bulletin</td>
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<td></td>
<td>Calculation errors</td>
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<td>Unanticipated results</td>
<td>Samples measured at incorrect wavelength</td>
<td>Check the equipment and filter settings</td>
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<tr>
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<td>Samples contain interfering substances</td>
<td>If possible, dilute sample further</td>
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<tr>
<td></td>
<td>Sample readings above/below the linear range</td>
<td>Concentrate or dilute samples so readings are in the linear range</td>
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</tbody>
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