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Product Information

Magnesium Assay Kit

Catalog Number **MAK026**
Storage Temperature –20 °C

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 Mg^{2+}, 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 Fe^{2+}, Cu^{2+}, Ni^{2+}, Zn^{2+}, Co^{2+}, Ca^{2+}, and Mn^{2+}.

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

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

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.

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.

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

Reagent	**Volume**
Magnesium Assay Buffer	35 µL
Developer	10 µL
Magnesium Enzyme Mix	5 µL

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 $(A_{450})_{initial}$ value from the $(A_{450})_{final}$ 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

$$S_a/S_v = C$$

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 nmole/50 μL = 0.1168 nmole/μL

0.1168 nmole/μL $\times$ 24.3 ng/nmole = 2.84 ng/μL

Troubleshooting Guide

Problem	Possible Cause	Suggested Solution
Assay not working	Cold assay buffer	Assay Buffer must be at room temperature
	Omission of step in procedure	Refer and follow Technical Bulletin precisely
	Plate reader at incorrect wavelength	Check filter settings of instrument
	Type of 96 well plate used	For colorimetric assays, use clear plates
Samples with erratic readings	Samples prepared in different buffer	Use the Assay Buffer provided or refer to Technical Bulletin for instructions
	Cell/Tissue culture samples were incompletely homogenized	Repeat the sample homogenization, increasing the length and extent of homogenization step.
	Samples used after multiple freeze-thaw cycles	Aliquot and freeze samples if needed to use multiple times
	Presence of interfering substance in the sample	If possible, dilute sample further
	Use of old or inappropriately stored samples	Use fresh samples and store correctly until use
Lower/higher readings in samples and standards	Improperly thawed components	Thaw all components completely and mix gently before use
	Use of expired kit or improperly stored reagents	Check the expiration date and store the components appropriately
	Allowing the reagents to sit for extended times on ice	Prepare fresh Master Reaction Mix before each use
	Incorrect incubation times or temperatures	Refer to Technical Bulletin and verify correct incubation times and temperatures
	Incorrect volumes used	Use calibrated pipettes and aliquot correctly
Non-linear standard curve	Use of partially thawed components	Thaw and resuspend all components before preparing the reaction mix
	Pipetting errors in preparation of standards	Avoid pipetting small volumes
	Pipetting errors in the Reaction Mix	Prepare a Master Reaction Mix whenever possible
	Air bubbles formed in well	Pipette gently against the wall of the plate well
	Standard stock is at incorrect concentration	Refer to the standard dilution instructions in the Technical Bulletin
	Calculation errors	Recheck calculations after referring to Technical Bulletin
	Substituting reagents from older kits/lots	Use fresh components from the same kit
Unanticipated results	Samples measured at incorrect wavelength	Check the equipment and filter settings
	Samples contain interfering substances	If possible, dilute sample further
	Sample readings above/below the linear range	Concentrate or dilute samples so readings are in the linear range

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