Product Manual

Magnesium Assay Kit

Catalog Number

MET-5208 **100 assays**

FOR RESEARCH USE ONLY
Not for use in diagnostic procedures

Introduction

Magnesium is a chemical element that forms several different compounds such as magnesium chloride, magnesium citrate, magnesium hydroxide (milk of magnesia), and magnesium sulfate heptahydrate (Epsom salts). Magnesium, through its interaction with phosphate, is essential to the basic nucleic acid chemistry of all cells of all known living organisms. Over 300 enzymes need magnesium ions to catalyze reactions, including all enzymes using or synthesizing ATP as well as those that use other nucleotides to synthesize DNA and RNA. The ATP molecule is typically found in a binding interaction with a magnesium ion.

Cell Biolabs' Magnesium Assay Kit is a simple assay for measuring magnesium levels in biological samples. Magnesium levels may be quantified in a wide range of biological samples including serum, plasma and urine. The kit has a detection sensitivity limit of 7.8 µM magnesium. Each kit provides sufficient reagents to perform up to 100 assays*, including standard curve and unknown samples.

Note: Each sample replicate requires 2 assays, one treated with glycerol kinase (+GK) and one without (-GK). The magnesium level is calculated from the difference in fluorescence readings from these 2 assays.

Assay Principle

The Magnesium Assay Kit is a sensitive quantitative fluorometric assay for magnesium. Glycerol kinase converts glycerol and ATP to glycerol-3-phosphate and ADP in a magnesium dependent manner. Glycerol-3-phosphate oxidase converts glycerol-3-phosphate and oxygen to dihydroxyacetone phosphate and hydrogen peroxide. The hydrogen peroxide is then detected with a highly specific fluorometric probe. Horseradish peroxidase catalyzes the reaction between the probe and hydrogen peroxide, which bind in a 1:1 ratio. Samples are compared to a known concentration of magnesium standard within the 96-black well microtiter plate format. Samples and standards are incubated for 5-10 minutes and then read with a standard 96-well fluorometric plate reader (Figure 1).

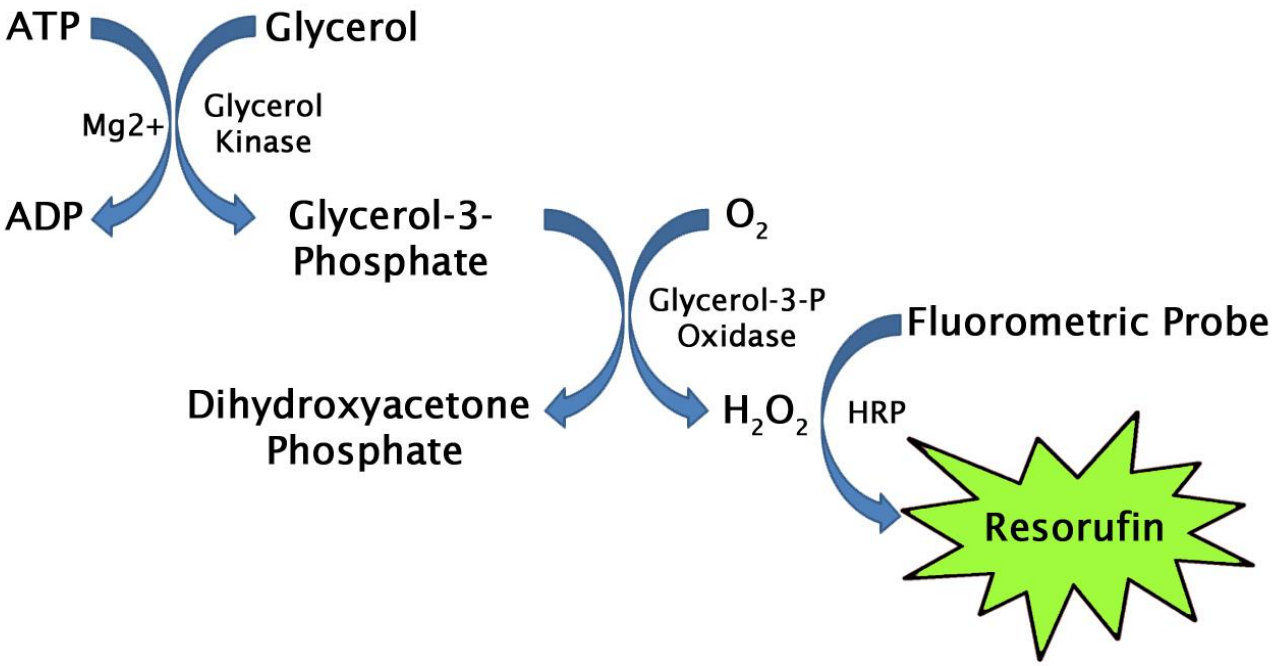

Figure 1. Assay Principle.

Related Products

1. MET-5054: L-Amino Acid Assay Kit
2. MET-5056: Branched Chain Amino Acid Assay Kit
3. MET-5071: Taurine Assay Kit
4. MET-5163: ATP Assay Kit
5. MET-5029: Pyruvate Assay Kit

Kit Components (shipped on dry ice)

1. $MgCl_2$ Standard (Part No. 52081A): One 50 µL vial of $MgCl_2$ at 50 mM
2. 10X Assay Buffer (Part No. 52082D): One 1.5 mL vial
3. Glycerol Substrate (Part No. 52083A): One 1 mL vial
4. 5X Enzyme Mixture (Part No. 239803): Four 525 µL vials
5. 5X Negative Control Mixture (Part No. 52084D): Four 525 µL vials
6. 200X Fluorometric Probe (Part No. 239901): One 55 µL vial

Materials Not Supplied

1. Distilled or deionized water
2. 96 well black plate
3. Microplate Fluorometer

Storage

Upon receipt, store the $MgCl_2$ Standard and Glycerol Substrate at room temperature. Store all other components at -80ºC. Avoid multiple freeze/thaw cycles. The 200X Fluorometric Probe is light sensitive and must be stored protected from light.

Preparation of Reagents

Note: All reagents must be brought to room temperature prior to use.

- 10X Assay Buffer, 5X Enzyme Mixture, and 5X Negative Control Mixture should be thawed/maintained at 4ºC during assay preparation. For longer term storage, each should be aliquoted and frozen at -80ºC to avoid multiple freeze/thaws.
- 200X Fluorometric Probe should be thawed/maintained at room temperature during assay preparation. Any unused material should be aliquoted and frozen at -80ºC to avoid multiple freeze/thaws.
- Reaction Reagent: Prepare the Reaction Reagent solution by diluting the kit components accordingly. Dilute the 10X Assay Buffer 1:10, 5X Enzyme Mixture 1:5, and Fluorescence Probe 1:100 in deionized water. See Table 1A below for examples of Reaction Reagent preparation based on the number of assays employed.

  Note: Maintain all components and mixtures at 4ºC. Prepare the Reaction Reagent solution sequentially based on the chart below, beginning with the Deionized Water and adding each component to the mixture. Add the Fluorescence Probe last to the solution. Vortex the solution thoroughly between each component addition, and protect the solution from light until use. Prepare only enough for immediate use by scaling the chart examples proportionally based on the number of assays needed. For best results, place the Reaction Reagent on ice and use within 30 minutes of preparation. Do not store Reaction Reagent solutions. The Reaction Reagent can appear slightly pink in color; this is normal background and should be subtracted from all absorbance values.

Deionized Water (mL)	10X Assay Buffer (mL)	5X Enzyme Mixture (mL)	200X Fluorescence Probe (µL)	Number of Assays	Total Volume (80 µL/well)
2.975	0.5	1	25	**50**	**4500**
1.190	0.2	0.4	10	**20**	**1800**

Table 1A. Preparation of Reaction Reagent.

- Negative Control Reagent: Prepare the Negative Control Reagent solution by diluting the kit components accordingly. Dilute the 10X Assay Buffer 1:10, 5X Negative Control Mixture 1:5, and Fluorescence Probe 1:100 in deionized water. See Table 1B below for examples of Negative Control Reaction Mixture preparation based on the number of assays employed.

  Note: Maintain all components and mixtures at 4ºC. Prepare the Negative Control Reaction Reagent solution sequentially based on the chart below, beginning with the Deionized Water and adding each component to the mixture. Add the Fluorescence Probe last to the solution. Vortex the solution thoroughly between each component addition, and protect the solution from light until use. Prepare only enough for immediate use by scaling the chart examples proportionally based on the number of assays needed. For best results, place the Negative Control Reaction Reagent on ice and use within 30 minutes of preparation. Do not store Negative Control Reaction Reagent solutions. The Reaction Reagent can appear slightly pink in color; this is normal background and should be subtracted from all absorbance values.

Deionized Water (mL)	10X Assay Buffer (mL)	5X Negative Control Mixture (mL)	200X Fluorescence Probe (µL)	Number of Assays	Total Volume (80 µL/well)
2.975	0.5	1	25	**50**	**4500**
1.190	0.2	0.4	10	**20**	**1800**

Table 1B. Preparation of Negative Control Reaction Reagent.

Preparation of Samples

- Cell lysates: Resuspend cells in distilled water. Homogenize or sonicate the cells on ice (to prevent vessel damage). Centrifuge 10,000 x g for 10 minutes at 4°C to remove debris. Collect the supernatant. The supernatant may be assayed undiluted or diluted as necessary into distilled water.
- Plasma: Collect blood with an anticoagulant such as heparin, citrate or EDTA and mix by inversion. Centrifuge the blood at 1000 x g at 4°C for 10 minutes. Collect plasma supernatant without disturbing the white buffy layer. Sample should be tested immediately or frozen at -80°C for storage. Plasma may need to be diluted into distilled water before assaying in order to detect magnesium in the standard curve range.
- Serum: Collect blood in a tube with no anticoagulant. Allow the blood to clot at room temperature for 30 minutes. Centrifuge at 2500 x g for 20 minutes. Remove the yellow serum supernatant without disturbing the white buffy layer. Samples should be tested immediately or frozen at -80°C for storage. Serum may need to be diluted into distilled water before assaying in order to detect magnesium in the standard curve range.
- Urine: To remove insoluble particles, centrifuge at 10000 x g for 10 min at 4°C. Collect the supernatant. The supernatant can be assayed directly or diluted as necessary into distilled water. Samples should be tested immediately or frozen at -80°C for storage. Urine may need to be diluted into distilled water before assaying in order to detect magnesium in the standard curve range.

Notes:

1. *Samples with NADH concentrations above 10 µM and glutathione concentrations above 50 µM will oxidize the Fluorescence Probe and could result in erroneous readings. To minimize this interference, it is recommended that superoxide dismutase (SOD) be added to the reaction at a final concentration of 40 U/mL.*
2. *Avoid samples containing DTT or β-mercaptoethanol since the Fluorescence Probe is not stable in the presence of thiols (above 10 µM).*
3. *The Fluorescence Probe is unstable at high pH (>8.5).*

Preparation of Standard Curve

Prepare fresh Magnesium standards before use by diluting in deionized water according to Table 1 below.

Standard Tubes	50 mM $MgCl_2$ Solution (µL)	Deionized Water (µL)	$MgCl_2$ (µM)
1	5	495	500
2	250 of Tube #1	250	250
3	250 of Tube #2	250	125
4	250 of Tube #3	250	62.5
5	250 of Tube #4	250	31.3
6	250 of Tube #5	250	15.6
7	250 of Tube #6	250	7.8
8	0	250	0

Table 2. Preparation of $MgCl_2$ Standards

Assay Protocol

Each $MgCl_2$ standard and sample should be assayed in duplicate or triplicate. A freshly prepared standard curve should be used each time the assay is performed.

Important Note: Each sample replicate requires two paired wells, one to be treated with Glycerol Kinase (present in the 5X Enzyme Mixture) and one without the enzyme (absent in the 5X Negative Control Mixture).

1. Add 10 µL of the diluted $MgCl_2$ standards or samples to the 96-well fluorescence microtiter plate.
2. Add 10 µL of Glycerol Substrate to each well.
3. Add 80 µL of the Reaction Reagent (see Preparation or Reagents section) to each standard well and one half of the paired sample wells and mix thoroughly.
4. Add 80 µL of the Negative Control Reaction Reagent (see Preparation of Reagents section) to the remaining half of the paired sample wells and mix thoroughly.
5. Cover the plate wells to protect the reaction from light.
6. Incubate at room temperature for 5-10 minutes on an orbital shaker.
7. Read the plate with a fluorescence microplate reader equipped for excitation in the 530-560 nm range and for emission in the 585-595 nm range.

8. Calculate the concentration of magnesium within samples by comparing the sample fluorescence to the standard curve as described below in the calculation of results section.

Calculation of Results

1. Determine the average Relative Fluorescence Unit (RFU) values for each sample, control, and standard.
2. Subtract the average zero standard value from itself and all standard values.
3. Graph the standard curve (see the example in Figure 2).
4. Subtract the sample well values without Glycerol Kinase (-GK) from the sample well values containing Glycerol Kinase (+GK) to obtain the difference. The fluorescence difference is due to the Glycerol Kinase activity.

   Net RFU = (RFU$_{+GK}$) - (RFU$_{-GK}$)

5. Compare the net RFU of each sample to the standard curve to determine and extrapolate the quantity of magnesium present in the sample. Only use values within the range of the standard curve.

Example of Results

The following figures demonstrate typical Magnesium Assay results. One should use the data below for reference only. This data should not be used to interpret actual results.

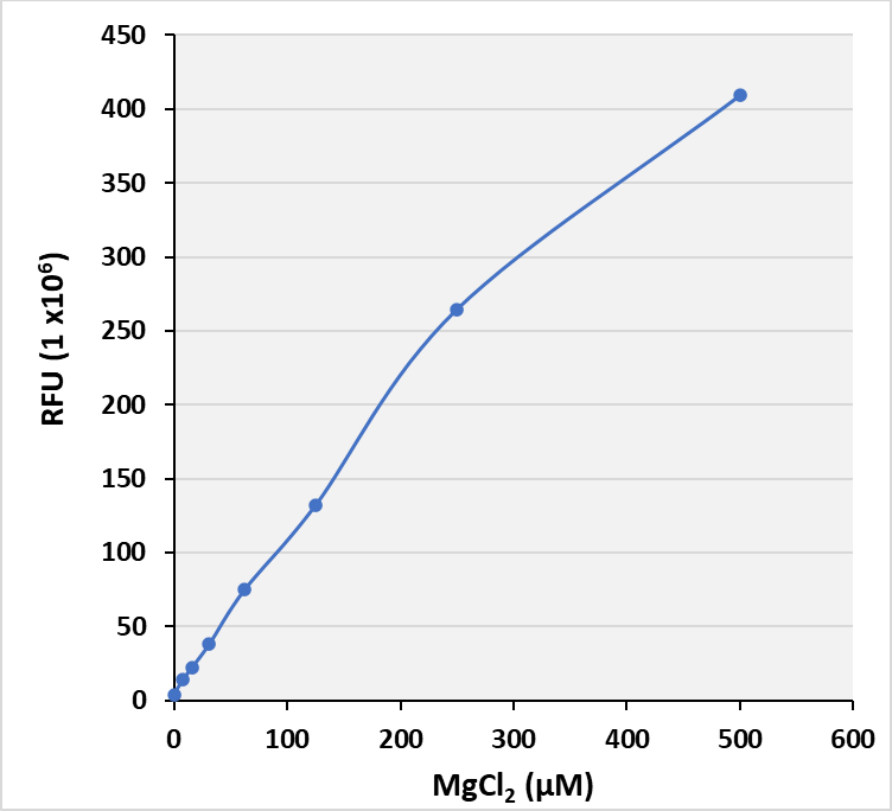

Figure 2. Example $MgCl_2$ Standard Curve.

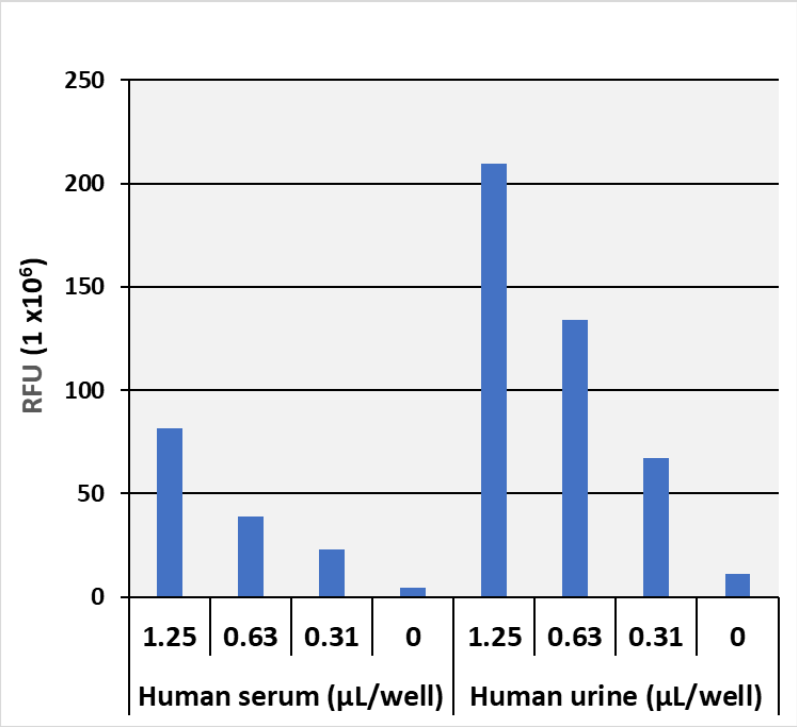

Figure 3. Detection of Mg2+ in human serum or urine.

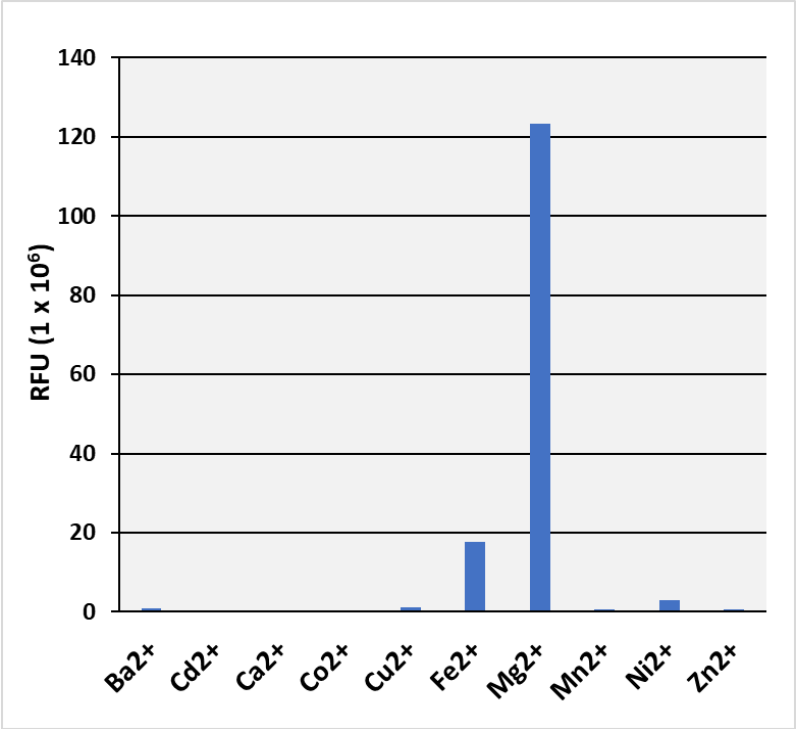

Figure 4. Specificity of Magnesium Assay Kit. Reactions were performed in the presence of 125 µM $BaCl_2$ (Ba^{2+}), $CdSO_4$ (Cd^{2+}), $CaCl_2$ (Ca^{2+}), $CoCl_2$ (Co^{2+}) $CuSO_4$ (Cu^{2+}), $FeSO_4$ (Fe^{2+}), $MgCl_2$ (Mg^{2+}), $MnCl_2$ (Mn^{2+}), $NiSO_4$ (Ni^{2+}), or $ZnCl_2$ (Zn^{2+}).

References

1. Arnaud MJ (2008). *Br. J. Nutr*. 99: S24 – S36.
2. Votyakova TV, and Reynolds IJ (2001) *Neurochem*. **79**:266.
3. Wester PO (1987). *Am. J. Clin. Nutr*. 45:1305–1312.
4. Romani, Andrea, M.P. (2013). In Astrid Sigel; Helmut Sigel; Roland K. O. Sigel (eds.). *Interrelations between Essential Metal Ions and Human Diseases*. Metal Ions in Life Sciences. 13:49–79.
5. Saris NE, Mervaala E, Karppanen H, Khawaja JA, Lewenstam A (2000). *Clin Chim Acta.* 294: 1–26
6. Rob PM, Dick K, Bley N, Seyfert T,; Brinckmann C, Höllriegel V; Friedrich HJ, Dibbelt L, and Seelig MS (1999). *J. Intern. Med*. 246: 373–378.
7. Baselt, R.(2008). *Disposition of Toxic Drugs and Chemicals in Man* (8th ed.). Biomedical Publications. pp. 875–877

Warranty

These products are warranted to perform as described in their labeling and in Cell Biolabs literature when used in accordance with their instructions. THERE ARE NO WARRANTIES THAT EXTEND BEYOND THIS EXPRESSED WARRANTY AND CELL BIOLABS DISCLAIMS ANY IMPLIED WARRANTY OF MERCHANTABILITY OR WARRANTY OF FITNESS FOR PARTICULAR PURPOSE. CELL BIOLABS' sole obligation and purchaser's exclusive remedy for breach of this warranty shall be, at the option of CELL BIOLABS, to repair or replace the products. In no event shall CELL BIOLABS be liable for any proximate, incidental or consequential damages in connection with the products.

Contact Information

Cell Biolabs, Inc.
5628 Copley Drive
San Diego, CA 92111
Worldwide: +1 858-271-6500
USA Toll-Free: 1-888-CBL-0505
E-mail: tech@cellbiolabs.com
www.cellbiolabs.com