Product Information

Magnesium Colorimetric Assay Kit

I. Kit Contents:

Components	K2068-100 100 assays	Cap Color	Part Number
Magnesium Assay Buffer	25 ml	WM	K2068-C-1
Magnesium Developer	lyophilized	Red	K2068-C-2
Magnesium Enzyme Mix	lyophilized	Green	K2068-C-3
Magnesium Standard (150 nmol/µl)	0.1 ml	Yellow	K2068-C-4

II. Introduction:

Magnesium is the 11th most abundant element in the human body. Mg^{2+} is essential for all living cells where it is involved in facilitating the processing of biological polyphosphates like DNA, RNA, ATP and enzyme functions. Mg^{2+} is the metallic ion at the center of chlorophyll. Mg^{2+} can be used as antacids and laxatives, and used to stabilize blood vessel spasm such as eclampsia and abnormal nerve excitation.

The Magnesium Colorimetric Assay Kit provides a simple and sensitive way for detection of magnesium concentration in various biological samples based on colorimetric method and the specific requirement of glycerol kinase for Mg^{2+}. The enzyme linked reaction generates an intensely colored (λmax = 450nm) product whose formation is proportional to Mg^{2+} concentration. The colored product can be easily qualified using a microtiter plate reader or a spectrophotometer at 450 nm. The linear range is 2 - 15 nmoles with detection sensitivity ~ 40 µM.

III. Storage and Handling:

Store kit at –20°C protect from light. Warm buffer to room temperature before use. Briefly centrifuge all small vials prior to opening.

Reagent Preparation:

Developer: Dissolve with 1.1 ml dH_2O. 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 supplied. 150 nmol/µl of Mg^{+2} Standard stock solution. Store at –20°C Mix before each use.

IV. Magnesium Assay Protocol:

1. Standard Curve Preparations:

Dilute the standard to 1.5 nmol/µl by adding 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: Tissue or cells can be extracted with 4 volume of Magnesium Assay Buffer, spin 16000g 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. Normal serum contains Mg^{2+} 0.7-1.05 mM (1.65-2.55 mg/dL), use 5 µl serum for testing. Urine should be diluted 10X. 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

4. Add 50 µl of the Reaction Mix to each well containing the Magnesium Standard and test samples. For best results, use a multichannel pipettor to initiate reaction in all samples at the same time. Mix well.

5. Incubate at 37°C for 10 mim. Read the plate OD_{450nm} to get A0 for each standard or Sample.

Notes:

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

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

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

6. Incubate the reaction for additional 10-30 min, read the OD again to get reading A. We recommend monitor the reaction kinetics to ensure the readings are in linear range when read the plate for the additional 10-30 minutes. All readings should not exceed 1.5 OD.

7. Calculation: Subtract A0 from standard and sample readings to get ΔOD = A-A0. Plot Magnesium standard curve. Apply sample ΔOD to the standard curve to get Mg^{2+} amount B (nmol) in the reaction well. Mg^{2+} concentration:

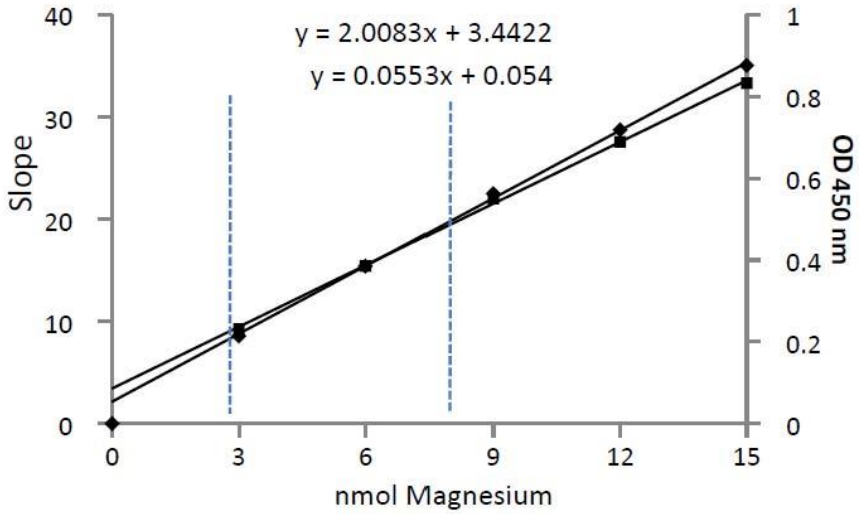
C = B/V (nmol/ml or µM)

Where: B is Mg^{2+} 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.

The assay may also be calculated by monitoring reaction slopes in the standards and samples reaction.



Magnesium standard curve: Assay is performed according to kit protocol. Vertical dotted lines indicate the lower and upper limits of normal serum Mg^{2+} concentrations.

V. Frequently Asked Questions

1. I am looking for a test kit for the quantitative determination of magnesium in EGTA-masked samples and photometric measurement should be made as quickly as possible. My sample is some DNA, some RNA and some enzymes and proteins. Is this Kit suitable for my experiment?

Yes, this assay kit would most definitely be suitable for any biological samples they are planning on using. When you say DNA/RNA samples, are these purified samples. In such case does the client know that there will be free Mg, (other than what is present in the solvent in this solution) to be assayed? we just want to be sure of this, before they start using the kit.

2. I would like to know that after “Magnesium Standard” was once thawed, the aliquot of “Magnesium Standard” can be stored at -20℃ again until the next assay? I would like to know wheather the 96 well plates used in this assay should be flat bottom or U bottom?Should this assay be done in duplicate?

Yes, once thawed, the standard can be stored in aliquots at -20℃.

You can use a flat bottom plate for the assay.

Doing the assay in triplicates would be best, but if that is not possible, duplicates are required.

3. I bought the magnesium assay kit from ApexBio last month and used it to measure Mg++ concentration for my acidified biological samples. All these samples are supposed to have substantial amount of magnesium even after dilution but the absorbance of all samples turned out to be near the blank value. The standards were fine by the way. I’m wondering if this kit is not intended to be used for acidified samples?

This is an enzyme based assay which is dependent of the pH of the assay buffer that is provided in the kit. Hence, if the samples are quite acidic it was hamper the reaction mechanisms of the kit.

4. Can this kit be used with samples like bacteria, plants, drosophila, yeast etc?

We have optimized the kit with mammalian samples. However, theoretically these kits should work with samples from multiple species/sources. Since the optimal conditions depend on the sample type, the protocol has to be be adapted to fit the samples for efficient results. Please refer to this kits citations to see what kind of samples have been used with this kit other than mammalian samples.

5. Can we use frozen samples with this assay?

Fresh samples are always preferred over frozen samples. However, frozen samples can also be used, provided, they were frozen right after isolation, were not freeze thawed multiple time (for which we recommend aliquoting the samples before freezing) and have been frozen for relatively short periods.

6. Can we use a different wavelength than recommended for the final analysis?

It is always recommended to use the exact recommended wavelength for the most efficient results. However, most plate readers have flexibility in their band width of detection in increments of +/- 10 nm. Depending on this flexibility range, you can deviate from the recommended wavelengths within limits.

7. What is the exact volume of sample required for this assay?

There is no specific volume we can recommend for the amount any sample to be used since it is completely sample concentration and quality based. You have to do a pilot expt with multiple sample volumes to determine the optimal volume which gives a reading within the linear range of the standard curve. Please refer to the citations for this product to see what other clients have used with similar sample types.

8. What is the shelf life of this kit?

This kit is good for 12 months from the date of shipment in the unopened form when stored at the appropriate temperature and appropriate conditions. After opening and reconstitution, some of the components in this kit are good for 2 months at -20℃. Please refer to the datasheet for storage information and shelf life of each of the components.

9. Why are my standard curve values lower than those shown on the datasheet?

There are multiple factors which influence the signals like the incubation times, room temperature, handling etc. In general, to increase the value of the standards, you can increase the incubation time. As long as the standard curve is linear, it should be fine to use, since all of your samples will also be measured under the same conditions on this curve.

10. How do I normalize my samples against protein concentration?

You can use a protein quantitation assay on the supernatants you get from cell/tissue lysates or with any other liquid sample in the assay buffer.

11. Can we use an alternate buffer for sample preparation (cell lysis, sample dilutions etc)?

Our assay buffers are optimized for the reactions they are designed for. They not only contain some detergents for efficient lysis of your cells/tissue, but also contain some proprietary components required for the further reactions. Therefore, we highly recommend using the buffers provided in the kit for the best results.

12. Should I make a standard curve for every expt I do, or is one curve/kit enough?

Yes, I would strongly recommend you to do the standards every time you do the expt. There is always a chance that something was done differently that day and we do not want any conditions to differ between standards and samples.

For research use only! Not to be used in humans.

Our promise

If the product does not perform as described on this datasheet, we will offer a refund or replacement. For more details, please visit http://www.apexbt.com/ or contact our technical team.

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