

Magnesium Colorimetric Assay Kit

For quantitative determination of Magnesium concentration in biological samples.

This product is for research use only and is not intended for diagnostic use.

1. Overview

Magnesium Assay Kit is a simple, direct and automation-ready procedure for measuring magnesium concentration in biological samples. This assay kit is designed to measure magnesium directly in biological samples without any pretreatment. A calmagite dye in the kit forms a colored complex specifically with magnesium. The intensity of the color, measured at 500 nm, is directly proportional to the magnesium concentration in the sample. The optimized formulation minimizes interference by potential substances.

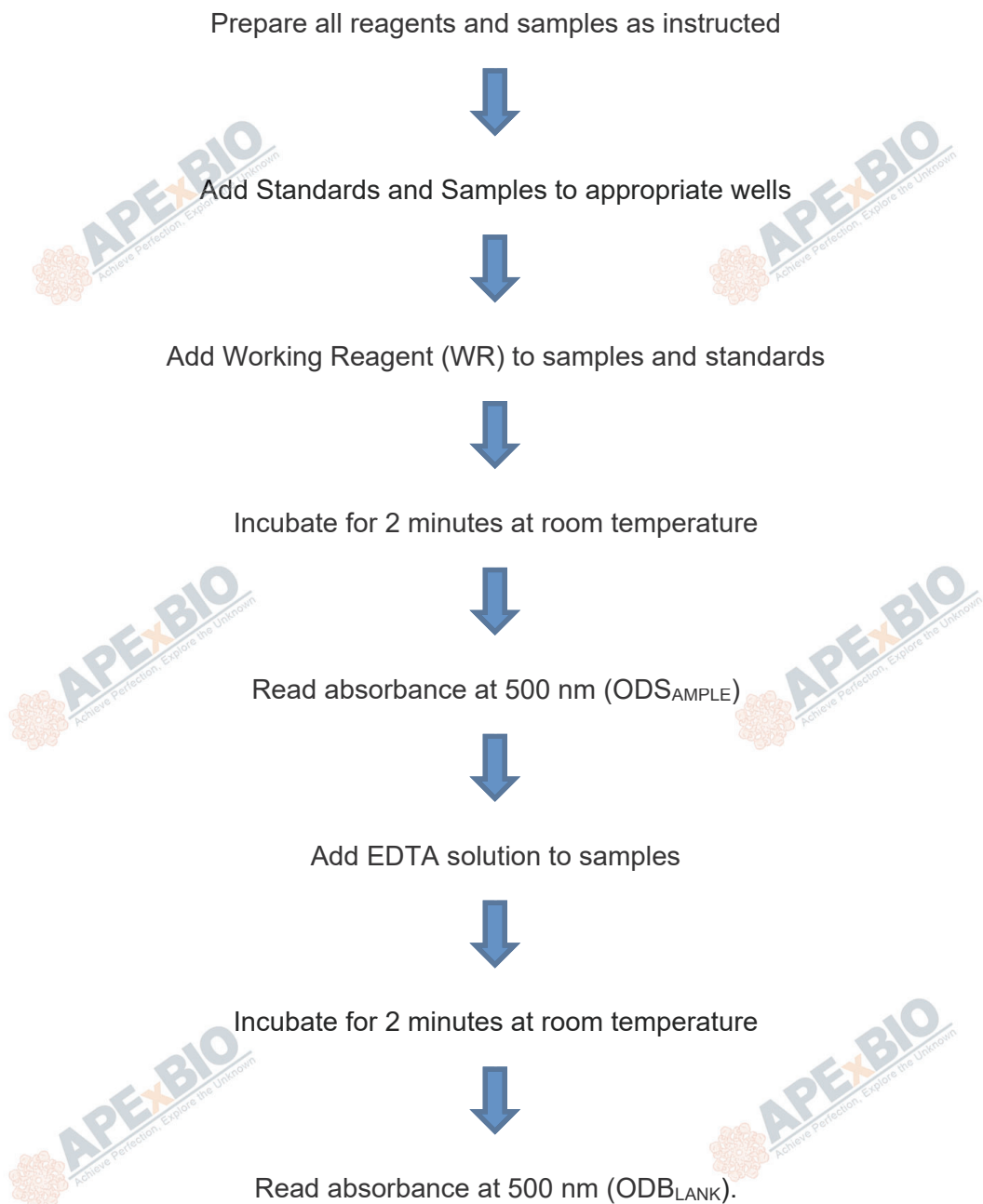
Sensitive and accurate: Use as little as 5 μ L sample. Linear detection range 0.1 mg/dL (41 μ M) to 3 mg/dL (1.2 mM) Mg^{2+} in 96-well plate assay.

Simple and high-throughput: The procedure involves addition of two reagents and measuring OD500nm. Can be readily automated as a high-throughput assay for thousands of samples per day.

Improved reagent stability and versatility: The optimized formulation has greatly enhanced reagent and signal stability. Cuvette or 96-well plate assay.

Low interference in biological samples: Assays can be directly performed in serum and urine samples.

2. Protocol Summary



3. Precautions

Please read these instructions carefully prior to beginning the assay.

- All kit components have been formulated and quality control tested to function successfully as a kit.
- We understand that, occasionally, experimental protocols might need to be modified to meet unique experimental circumstances. However, we cannot guarantee the performance of the product outside the conditions detailed in this protocol booklet.
- Reagents should be treated as possible mutagens and should be handled with care and disposed of properly. Please review the Safety Datasheet (SDS) provided with the product for information on the specific components.

- Observe good laboratory practices. Gloves, lab coat, and protective eyewear should always be worn. Never pipet by mouth. Do not eat, drink or smoke in the laboratory areas.
- All biological materials should be treated as potentially hazardous and handled as such. They should be disposed of in accordance with established safety procedures.

4. Storage and Stability

Store kit at 4°C immediately upon receipt. Kit has a storage time of 12 months from receipt.

Refer to list of materials supplied for storage conditions of individual components. Observe the storage conditions for individual prepared components in the Materials Supplied section.

5. Limitations

- Assay kit intended for research use only. Not for use in diagnostic procedures.
- Do not mix or substitute reagents or materials from other kit lots or vendors. Kits are QC tested as a set of components and performance cannot be guaranteed if utilized separately or substituted.

6. Materials Supplied

Item	Quantity	Storage Condition
Reagent A	25 mL	+4°C
Reagent B	25 mL	+4°C
Magnesium Standard (10 mg/dL)	1 mL	+4°C
EDTA (0.1 M)	2 x 1.5 mL	+4°C

7. Materials Required, Not Supplied

These materials are not included in the kit, but will be required to successfully perform this assay:

- Distilled H₂O
- Multi-channel pipette
- 1.5 mL tubes
- 1.5 mL centrifuge
- 96-well clear plate with flat bottom (alternatively, 1 mL cuvettes may be used)
- Standard microplate reader - capable of reading absorbance at 500 nm

8. Technical Hints

- This kit is sold based on number of tests. A 'test' simply refers to a single assay well. The number of wells that contain sample, control or standard will vary by product. Review the protocol completely to confirm this kit meets your requirements. Please contact our Technical Support staff with any questions.
- Pre-rinse the pipette tip with the reagent, use fresh pipette tips for each sample, standard and reagent.
- Pipette standards and samples to the bottom of the wells.
- Add the reagents to the side of the tube to avoid contamination.
- Some Solutions supplied in this kit are caustic; care should be taken with their use.

9. Reagent Preparation

- Equilibrate all reagents to room temperature (18-25°C) prior to use.
- The kit contains enough reagents for 250 assays.

All reagents are supplied ready to use.

10. Standard Preparation

- Always prepare a fresh set of standards for every use.
- Prepare serially diluted standards immediately prior to use.

Dilute Standard:

10.1.1 Prepare 200 μ L of 2 mg/dL Standard by mixing 40 μ L of 10 mg/dL Standard and 160 μ L distilled water.

10.1.2 Diluted standard can be stored at 4°C for future use.

11. Sample Preparation

Sample treatment:

Assays can be performed directly in serum and urine samples. Urine (typically 7.2 -10.8 mg/dL magnesium) will need diluting to bring them within the linear range of the assay (up to 2.5 mg/dL).

Pre-treatment for lipid/lipoprotein-rich samples (e.g. milk):

- 11.1 Mix equal volumes of sample and 10% trichloroacetic acid.
- 11.2 Incubate 5 min at room temperature and pellet precipitates for 2 min at 14,000 rpm in a table centrifuge.
- 11.3 Assay the supernatant (dilution factor = 2) using the standard assay procedure.

Δ Note: EDTA and other Mg^{2+} chelators interfere with this assay. This assay cannot be applied to plasma samples obtained with EDTA.

12. Assay Procedure

- Equilibrate all materials and prepared reagents to room temperature prior to use.
- We recommend that you assay all standards, controls and samples in duplicate.

Working Reagent:

- Immediately prior to starting the reaction, as detailed in the table 12.1.1 Prepare enough Working Reagent (WR) for all samples and standards by mixing per reaction well equal volumes of Reagent A and Reagent B.

Component	Working Reagent (μ L/reaction)
Reagent A	100
Reagent B	100

Procedure using 96-well plate:

- 12.1.2 Add 5 μ L of dilute Standard and each Sample to separate wells.
- 12.1.3 Add 200 μ L of the WR to each Sample and Standard well and tap plate to mix thoroughly.
- 12.1.4 Incubate for 20 min at room temperature.
- 12.1.5 Read OD at 500 nm.
- 12.1.6 Add 10 μ L EDTA Solution to all Sample wells and tap plate to mix thoroughly.
- 12.1.7 Incubate 2 min at room temperature.
- 12.1.8 Read OD at 500nm (OD_{BLANK}).

Δ Note: Use 96-well clear, flat-bottom plates.

Procedure using cuvette:

- Set up test tubes prior starting assay.
- 12.1.9 Add 25 μ L diluted Standard and Samples to appropriately labeled tubes.
- 12.1.10 Add 1000 μ L WR and vortex to mix.
- 12.1.11 Incubate for 2 min at room temperature.
- 12.1.12 Transfer to cuvette.
- 12.1.13 Read OD at 500nm.
- 12.1.14 Add 50 μ L EDTA solution, mix well.
- 12.1.15 Incubate 2 min at room temperature and read OD at 500 nm (OD_{BLANK}).

13. Calculations

Magnesium concentration of the sample is calculated as:

$$[\text{Mg}^{2+}] = \frac{\text{OD}_{\text{SAMPLE}} - \text{OD}_{\text{BLANK}}}{\text{OD}_{\text{MG}} - \text{OD}_{\text{MGBLANK}}} \times 2 \text{ (mg/dL)}$$

OD_{SAMPLE} is OD_{500nm} values of sample before EDTA addition.

OD_{BLANK} is OD_{500nm} values of sample after EDTA addition.

OD_{MG} is OD_{500nm} values of the standard (2 mg/dL) before EDTA addition.

OD_{MGBLANK} is OD_{500nm} values of the standard (2 mg/dL) after EDTA addition.

Conversions: 1 mg/dL Mg²⁺ equals 411 µM, 0.001% or 10 ppm.

14. Typical Data

Typical standard curve – data provided **for demonstration purposes only**. A new standard curve must be generated for each assay performed.

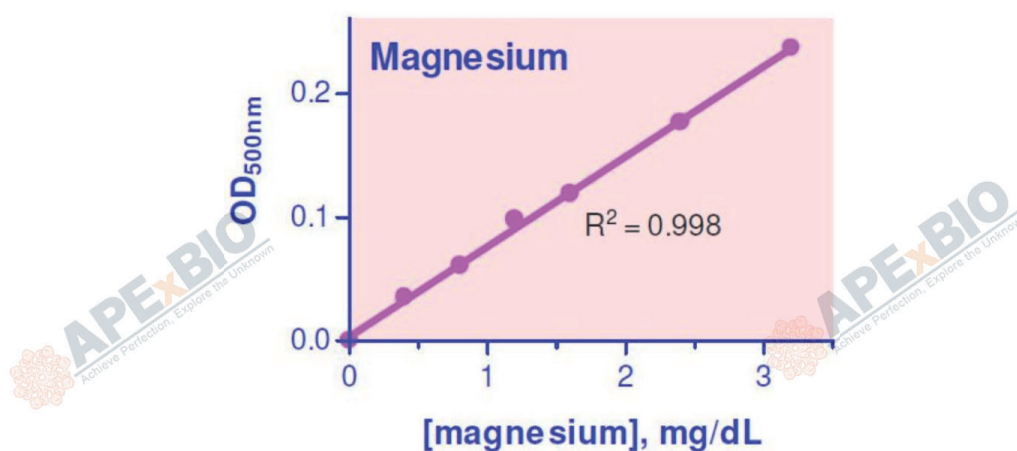


Figure 1. Example of Magnesium Assay Kit standard curve.

Typical values:

Samples were assayed in duplicate using the 96-well plate protocol. The Mg²⁺ values (mg/dL) were 1.64 ± 0.04 (rat serum), 1.77 ± 0.02 (human serum), 2.41 ± 0.5 (goat serum), 2.80 ± 0.14 (fetal bovine serum).

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

For more details, please visit <http://www.apexbt.com/> or contact our technical team.



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