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Catalog #CMG-035

MC Reagent Biochemical Assay Series

COSMO BIO CO., LTD.
Inspiration for Life Science

Magnesium Assay Kit

Xylidylblue-I Chromogenic method

Biochemical Significance and Test Summary

All ATP-dependent enzyme require Mg^{2+} as a cofactor for the enzymes to react and maintain the electrical excitability of the muscular and nervous cells. Regulation takes place mainly via the kidneys, especially via the ascending loop of Henle. A low magnesium level is found in malabsortion syndrome, diuretic or aminoglucoside therapy. Hypermagnesemia is found in acute and chronic renal failure, glomerulonephritis, Addisons's disease or intensive anti acid therapy, magnesium excess, and magnesium release from the intracellular space.
This product is a direct colorimetric assay kit without deproteinization of the sample. Magnesium with Xylidylblue-I (as chelator) at alkaline pH, yields a purple colored complex. The intensity of the color formed is proportional to the magnesium concentration in the sample.

1. Kit contents (100 tests)

R-1	Chelate color	1 x 25 mL	Ready to use
STD	2 mg/dL Mg Standard	1 x 0.5 mL	Ready to use

*Storage conditions: Store at 2-8°C. **Don't freeze**.
*Expiration: 1 year. After the vials are opened, the kit should be used within one month.
*Measuring range: 0.05-3.5 mg/dL

2. Materials required but not provided

(1) Distilled water
(2) Micropipettors and pipette tips
(3) Clear flat-bottom 96-well plate
(4) Microplate reader with 660 nm capability

3. Assay preparation

Bring all reagents to room temperature before use.

4. Sample preparation

Serum/ Plasma: Insoluble substances in serum and plasma samples should be removed by filtration or centrifugation. EDTA-plasma cannot be used.
Urine (24 hour pooled urine)/ Dilute the sample 1/2 in distilled water. Add 6M HCl to the diluted sample and adjust pH 2.0-3.0 (e.g. 5-10 µL of 6M HCl/1 mL of lysate). Centrifuge at 6,000 rpm for 15 minutes. Collect the supernatant and use it for assay.
Biological fluid: Add 6M HCl to the sample and adjust pH 2.0-3.0 (e.g. 5-10 µL of 6M HCl/1 mL of lysate). Centrifuge at 6,000 rpm for 15 minutes. Collect the supernatant and use it for assay.
Tissue: Add 5% TCA solution, vortex for 1 minute and incubate for 30 minutes at 4-8°C. Centrifuge at 6,000 rpm for 15 minutes. Collect the supernatant and use it for assay.

Note: Sample pH should be between pH 2 and pH 8.

5. Assay protocol

(1) Add 5 µL of Distilled water(Blank)/STD(Standard)/Sample into each well.
(2) Add 250 µL of R-1 to each well and incubate for 5 minutes at room temperature.
(3) Read the absorbance at 660 nm. ----- OD

6. Calculations

$\Delta OD_{Standard} = OD_{Standard} - OD_{Blank}$, $\Delta OD_{Sample} = OD_{Sample} - OD_{Blank}$

Magnesium (mg/dL) = $\Delta OD_{Sample} / \Delta OD_{Standard}$ x 2
Magnesium (mM) = $\Delta OD_{Sample} / \Delta OD_{Standard}$ x 0.824

(Assay example)

	OD (660 nm)	Δ OD	Magnesium (mg/dL)
DW (Blank)	0.387	-	-
Standard	0.241	-0.146	-
Sample	0.271	-0.116	1.59

$\Delta OD_{Standard} = 0.241 - 0.387 = -0.146$
$\Delta OD_{Sample} = 0.271 - 0.387 = -0.116$

$Magnesium_{Sample} = \Delta OD_{Sample} / \Delta OD_{Standard}$ x 2 = (-0.116/ -0.146) x 2 = 1.59 (mg/dL)
$Magnesium_{Sample}$ (mM) = $\Delta OD_{Sample} / \Delta OD_{Standard}$ x 0.824 = (-0.116/ -0.146) x 0.824 = 0.655 (mM)

7. Interferences

EDTA inhibits magnesium to chromogenic system. The test is not affected by presence of bilirubin-F and bilirubin-C up to 40 mg/dL, hemoglobin up to 1 g/dL and chyle up to 3000 FTU.

8. Quality Control

Use of control sera is recommended to monitor the quality of assay results.

9. Reference.

(1) Mann C. K, Yoe J. H : Spectrophotometric determination of magnesium with sodium 1-azo-2-haydroxy-3-(2,4-dimethyl-carboxanili-do)-naphtalene-1-(2-hydroxy-benzene-5-sulphonate), *Anal Chem*, 28, p202-205 (1956)
(2) Sakamoto. A, Terui. Y, Yamamoto. T, Kasahara. T, Nakamura. M, Tomitori. H, Yamamoto. K, Ishihama A, Michael. AJ, Igarashi. K, Kashiwagi. K : Enhanced biofilm formation and/or cell viability by polyamines through stimulation of response regulators UvrY and CpxR in the two-component signal transducing systems, and ribosome recycling factor, *Int J Biochem Cell Biol*, 44(15), p1877-1886 (2012).

10. Technical support & troubleshooting

(1) Unstableness of incubation temperature may result in unstable results.
(2) Use disposable test tube and glassware washed with 1M HNO_3 or 1M HCl, and rinse with distilled water.
(3) Accuracy to the microliter is important to obtain good results. Ensure maximum precision when pipetting.
(4) Temperature for the chromogenic reaction may affect the optical density. It may be necessary to adjust the reaction time depending on the room temperature.
(5) High concentration of proteins or lipid in cell lysate or in tissue extract may affect the observed value. Please remove them by ultrafiltration or centrifugation.
(6) 24 hour pooled urine: Dilute the sample 1/10 in distilled water. Mix. Multiply results by 10 (dilution factor) for assay sample.

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