Instruction manual

* FOR RESEARCH USE ONLY
* STORE AT 4℃ UPON ARRIVAL

Magnesium Assay kit LS

(Xylidyl blue-I Chromogenic method)

Description

All ATP-dependent enzymes require Mg^{2+} as a cofactor to reacts enzyme and maintains 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 malabsorption 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 Xylidyl blue-I (as chelator) at alkaline pH, yields a purple colored complex. The intensity of the colour formed is proportional to the magnesium concentration in the sample.

Kit contents

200 tests （Catalog # : MG01ME ）

R-R	Chelate color (Xylidyl blue-I) ●	50 mL×1
STD	Magnesium Standard 2 mg/dL ●	0.6 mL×1

Note

A) Unstableness of incubation temperature may result in unstable results.

B) Use disposable test tube and glassware washed with 1M HNO_3 or 1M HCl solution and distilled water.

C) Accuracy in pipetting volume for samples and reagents may affect the quality of assay. Please note that samples, standards and Working Reagent must be poured accurately µL level.

D) Temperature for chromogen reaction may affect optical density. Please try to extend or shorten chromogen reaction time depending on room temperature.

E) In the cell lysate or the tissue extract use as specimen, high concentration of proteins or lipid, may affect observed value. Please remove its by ultrafiltration or centrifugation.

Operation

1. Sample preparation

◇Serum or Plasma

Insoluble substances in serum and plasma samples should be removed by filtration or centrifugation. EDTA-plasma cannot be used.

◇Tissue extract, Lysate, Other samples.

Urine (24 hour pooled urine), or other biological fluid:

Add 6M HCl to the sample and adjust pH 2.0-3.0 (e.g. 5-10µL 6M HCl/ 1mL of lysate.). Centrifuge at 6,000 rpm for 15 min. Collect the supernatant and use it for assay.

Tissue:

Add 5% TCA solution, vortex 1 min. and incubate at 4-8°C for 30 min. Centrifuge at 6,000 rpm for 15 min. Collect the supernatant and use it for assay.

* Sample pH should be between pH2 to pH8

2. Assay preparation

Bring all reagents to room temperature before use.

3. Assay procedure.

Procedure using microplate reader.

(1 assay sample 253µL)

○Assay

(1) Add 3 µL of Distilled water (Blank) / STD (Standard)/ sample into each well.

(2) Add 250 µL of R-R to each well and incubate at room temperature for 5 min.

(3) Read the absorbance at 660 nm. --> OD

Add (µL)		Assay Sample: Blank OD_{Bl}	Standard OD_{Std}	Sample OD_S
1	Distilled water	3	-	-
	STD	-	3	-
	Assay sample	-	-	3
2	R-R	250	250	250
↓ Mix and incubate for 5 minutes at room temperature. Read the absorbance at 660 nm.				

○Calculations

$\Delta OD_{Std} = OD_{Std} - OD_{Bl}$

$\Delta OD_S = OD_S - OD_{Bl}$

Magnesium (mg/dL) = $\Delta OD_S/\Delta OD_{Std}$ X 2

Magnesium (mM) = $\Delta OD_S/\Delta OD_{Std}$ X 0.823

(Assay example)

	OD (660nm)	ΔOD	Magnesium (mg/dL)
Blank	0.406	-	-
Standard	0.312	-0.094	-
Sample	0.321	-0.085	1.81

ΔOD_{Std} = 0.312 – 0.406 = -0.094

ΔOD_S = 0.271 – 0.387 = -0.116

$Magnesium_{Sample}$ (mg/dL) = $\Delta OD_S/\Delta OD_{Std}$ x 2

= -0.085/ -0.094 x 2 = 1.81 (mg/dL)

$Magnesium_{Sample}$ (mM) = $\Delta OD_S/\Delta OD_{Std}$ x 0.823

= -0.085/ -0.094 x 0.823 = 0.744 (mM)

*In diluted sample of seminal fluid, multiply the result by dilution-factor.

Performance

Measuring range 0.2 – 5.0 mg/dL

Imprecision Imprecision was evaluated using commercially available quality control serum.

Within run

	Mean mg/dL	S.D	C.V %
Level 1	1.64	0.08	4.1
Level 2	3.08	0.08	2.7

Interferences No interference by the note of substances were observed.
Conjugated bilirubin and unconjugated bilirubin 40 mg/dL
Hemoglobin 1 g/dL Chyle 3,000 FTU

Expiration date and preservation conditions

Storage conditions: Store at 2-8°C. Don't freeze.

Expiration: 1 year from the date of manufacture. After the bottles are opened,the kit should be used in 1 month.

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-sulph onate), *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).

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