



Ca²⁺/Mg²⁺ ATPase Activity

Colorimetric Microplate Assay Kit

User Manual

Catalog # CAK1020

(Version 1.7F)

Detection and Quantification of Ca²⁺/Mg²⁺ ATPase activity in Urine,
Serum, Plasma, Tissue extracts, Cell lysate, Cell culture media and
Other biological fluids Samples.

For research use only. Not for diagnostic or therapeutic procedures.

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I. INTRODUCTION

$\text{Ca}^{2+}/\text{Mg}^{2+}$ ATPase is widely distributed in plants, animals, microbes and cells, can catalyze the hydrolysis of ATP, ADP and inorganic phosphate.

$\text{Ca}^{2+}/\text{Mg}^{2+}$ ATPase Activity Colorimetric Microplate Assay Kit provides a convenient tool for sensitive detection of $\text{Ca}^{2+}/\text{Mg}^{2+}$ ATPase activity in a variety of samples. In this assay, $\text{Ca}^{2+}/\text{Mg}^{2+}$ ATPase breaks down ATP to generate ADP and inorganic phosphate, ATP activity is determined by measuring the amount of inorganic phosphorus.

II.KIT COMPONENTS

Component	Volume	Storage
96-Well Microplate	1 plate	
Assay Buffer	30 ml x 4	4 °C
Substrate	Powder x 1	-20 °C
Activator	Powder x 1	4 °C
Inhibitor	Powder x 1	4 °C
Dye Reagent I	Powder x 1	4 °C
Dye Reagent II	Powder x 1	4 °C
Dye Reagent III	20 ml x 1	4 °C
Stop Solution	4 ml x 1	RT
Standard (5mmol/L)	1 ml x 1	4 °C
Plate Adhesive Strips	3 Strips	
Technical Manual	1 Manual	

III. MATERIALS REQUIRED BUT NOT PROVIDED

1. Microplate reader to read absorbance at 660 nm
2. Distilled water
3. Pipettor, multi-channel pipettor
4. Pipette tips
5. Mortar
6. Ice
7. Centrifuge

IV. REAGENT PREPARATION

Standard: Perform 2-fold serial dilutions of the top standard solution using distilled water to make the standard curve. The concentration of standard curve could be 5/2.5/1.25/0.625/0.312/0.156/0.078 mmol/L.

Substrate: Add 17 ml distilled water to dissolve before use. Keep in dark for immediate use or store at -20°C for 1 month after reconstitution.

Activator: Briefly centrifuge prior to opening. Add 1 ml distilled water to dissolve before use, store at 4 °C.

Inhibitor: Briefly centrifuge prior to opening. Add 1 ml distilled water to dissolve before use, store at 4 °C for 6 months.

Dye Reagent: Add 10 ml Dye Reagent III into Dye Reagent I and 2 ml Dye Reagent III into Dye Reagent II respectively to dissolve. Transfer all Dye Reagent II into Dye Reagent III, mix, then transfer all Dye Reagent I into Dye Reagent III (Must follow this step). The mixed Dye Reagent may store at -20°C for 2-3 weeks.

***Note:** It should be yellow. If colorless, the solution is failure. If blue, the solution is polluted. This solution should be prepared before use. It is best to use disposable plastic containers to prepare the solution in order to prevent phosphorus pollution.

Note: Divide into small aliquots to avoid repeated freeze-thaw cycles.

V. SAMPLE PREPARATION

1. For cell and bacteria samples

Collect cell or bacteria into centrifuge tube, discard the supernatant after centrifugation, add 1 ml Assay Buffer for 5×10^6 cell or bacteria, sonicate (with power 20%, sonicate 3s, interval 10s, repeat 30 times); centrifuged at 8000g 4°C for 10 minutes, take the supernatant into a new centrifuge tube and keep it on ice for detection.

2. For tissue samples

Weigh out 0.1 g tissue, homogenize with 1 ml Assay Buffer on ice, centrifuged at 8000g 4°C for 10 minutes, take the supernatant into a new centrifuge tube and keep it on ice for detection.

3. For red blood cell samples

Add heparin into the blood, centrifuged at 2000g 4 °C for 5 minutes. Discard the plasma and white blood cells. Wash the red blood cells with PBS for 3 times, discard the supernatant after centrifugation each time. Add 0.9 ml Assay Buffer into 0.1 ml red blood cells, mix well, and wait for 15 minutes at room temperature.

VI. ASSAY PROCEDURE

Add following reagents into the microcentrifuge tubes:

Reagent*	Sample**	Control**	Standard	Blank
Substrate	170µl	170µl	--	--
Sample	20µl	20µl	--	--
Inhibitor	--	10 µl		
Activator	10 µl	--		
Mix, put it in the oven, 37°C for 30 minutes.				
Stop Solution	40 µl	40 µl	--	--
Mix, centrifuged at 10000g, room temperature for 5 minutes. Add following reagents into the microplate				
Standard	--	--	20 µl	--
Distilled water	--	--	--	20 µl
Supernatant	20 µl	20 µl	--	--
Dye Reagent	180 µl	180 µl	180 µl	180 µl
Mix, room temperature for 30 minutes, record absorbance measured at 660nm.				

Note:

*It is best to use disposable plastic tube to avoid phosphorus pollution.

*Reagents must be added sequentially and should not be premixed prior to addition.

**For unknown samples, we recommend doing a pilot experiment & testing several doses to ensure the readings are within the standard curve range. If the enzyme activity is lower, please add more samples into the reaction system; or increase the reaction time; if the enzyme activity is higher, please dilute the sample, or decrease the reaction time.

**For colored samples, we recommend setting a parallel sample background control well with same volume of sample only. Other reagents were replaced by distilled water to the same total volume. Subtract the OD value of the sample background control from the OD value of the sample to correct for interference from the sample's own color.

VII. CALCULATION

Unit Definition: One unit of $\text{Ca}^{2+}/\text{Mg}^{2+}$ ATPase activity is defined as the enzyme generates $1\mu\text{mol}$ of PO_4^{3-} per minute.

1. According to the slope of the standard curve

$$\text{Activity} = \frac{(\text{OD}_{\text{Sample}} - \text{OD}_{\text{Control}}) - \text{Intercept}}{\text{Slope} \times T} \times n (\text{U/mL})$$

2. According to one point of the standard OD value and concentration

2.1 According to the protein concentration of sample

$$\text{Activity} = \frac{(C_{\text{Standard}} \times V_{\text{Standard}}) \times (\text{OD}_{\text{Sample}} - \text{OD}_{\text{Control}})}{(\text{OD}_{\text{Standard}} - \text{OD}_{\text{Blank}}) \times V_{\text{Sample}} \times C_{\text{Protein}} \times T} (\text{U/mg})$$

2.2 According to the quantity of cells or bacteria

$$\text{Activity} = \frac{(C_{\text{Standard}} \times V_{\text{Standard}}) \times (\text{OD}_{\text{Sample}} - \text{OD}_{\text{Control}})}{(\text{OD}_{\text{Standard}} - \text{OD}_{\text{Blank}}) \times N \times (V_{\text{Sample}} / V_{\text{Assay}}) \times T} (\text{U}/10^4)$$

2.3 According to the weight of sample

$$\text{Activity} = \frac{(C_{\text{Standard}} \times V_{\text{Standard}}) \times (\text{OD}_{\text{Sample}} - \text{OD}_{\text{Control}})}{(\text{OD}_{\text{Standard}} - \text{OD}_{\text{Blank}}) \times W \times (V_{\text{Sample}} / V_{\text{Assay}}) \times T} (\text{U/g})$$

Slope: the absorbance slope of standard curve

n: the dilution factor

C_{Protein} : the protein concentration of sample, mg/mL

W: the weight of total sample, g

N: the quantity of total cell or bacteria sample, 10^4

C_{Standard} : the concentration of standard, mmol/L = $\mu\text{mol/mL}$

V_{Standard} : the volume of standard in assay procedure, mL

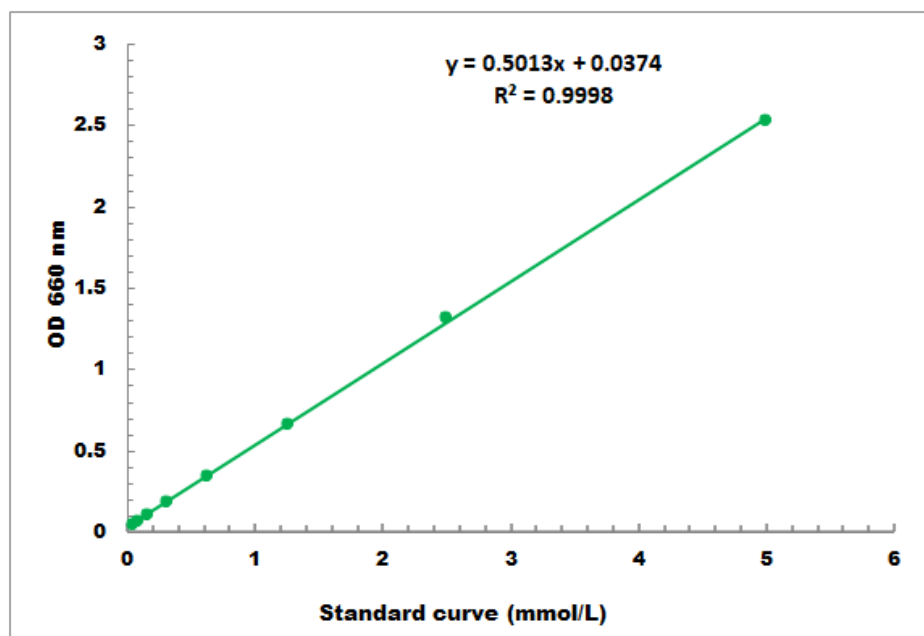
V_{Sample} : the volume of sample in assay procedure, mL

V_{Assay} : the volume of Assay Buffer in sample preparation, mL

T: the reaction time, minute

VIII. TYPICAL DATA

The standard curve is for demonstration only. A standard curve must be run with each assay.



Detection Range: 0.01 mmol/L - 5mmol/L