Ca^{2+}/Mg^{2+} ATPase Microplate Assay Kit

User Manual

Catalog # CAK1020

(Version 1.4F)

Detection and Quantification of Ca^{2+}/Mg^{2+} 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.

I. INTRODUCTION

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

Ca^{2+}/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 (5 µmol/ml)	1 ml x 1	4 °C
Plate Adhesive Strips	3 Strips	
Technical Manual	1 Manual	

Substrate: add 17 ml distilled water to dissolve before use, store at 4 °C.

Activator: add 1 ml distilled water to dissolve before use, store at 4 °C.

Inhibitor: add 1 ml distilled water to dissolve before use, store at 4 °C.

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.

1) Reagents must be added step by step, can not be mixed and added together.

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. 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.

V. ASSAY PROCEDURE

Add following reagents into the microcentrifuge tubes:

Reagent	Sample	Control	Standard	Blank
Substrate	170 µl	170 µl	--	--
Sample	20 µl	20 µl	--	--
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:

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

2) Perform 2-fold serial dilutions of the top standards to make the standard curve.

3) 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 sample into the reaction system; or increase the reaction time; if the enzyme activity is higher, please dilute the sample, or decrease the reaction time.

VI. CALCULATION

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

1. According to the protein concentration of sample

$$Ca^{2+}/Mg^{2+}\ ATPase\ (U/mg) = (C_{Standard} \times V_{Total}) \times (OD_{Sample} - OD_{Control}) / (OD_{Standard} - OD_{Blank}) / (V_{Sample} \times C_{Protein}) / T$$

$$= 2 \times (OD_{Sample} - OD_{Control}) / (OD_{Standard} - OD_{Blank}) / C_{Protein}$$

2. According to the weight of sample

$$Ca^{2+}/Mg^{2+}\ ATPase\ (U/g) = (C_{Standard} \times V_{Total}) \times (OD_{Sample} - OD_{Control}) / (OD_{Standard} - OD_{Blank}) / (W \times V_{Sample} / V_{Assay}) / T$$

$$= 2 \times (OD_{Sample} - OD_{Control}) / (OD_{Standard} - OD_{Blank}) / W$$

3. According to the concentration of cell or bacteria

$$Ca^{2+}/Mg^{2+}\ ATPase\ (U/10^4) = (C_{Standard} \times V_{Total}) \times (OD_{Sample} - OD_{Control}) / (OD_{Standard} - OD_{Blank}) / (N \times V_{Sample} / V_{Assay}) / T$$

$$= 2 \times (OD_{Sample} - OD_{Control}) / (OD_{Standard} - OD_{Blank}) / N$$

$C_{Protein}$: the protein concentration, mg/ml;

W: the weight of sample, g;

$C_{Standard}$: the concentration of Standard, 5 μmol/ml;

V_{Total}: the total volume of the enzymatic reaction, 0.24 ml;

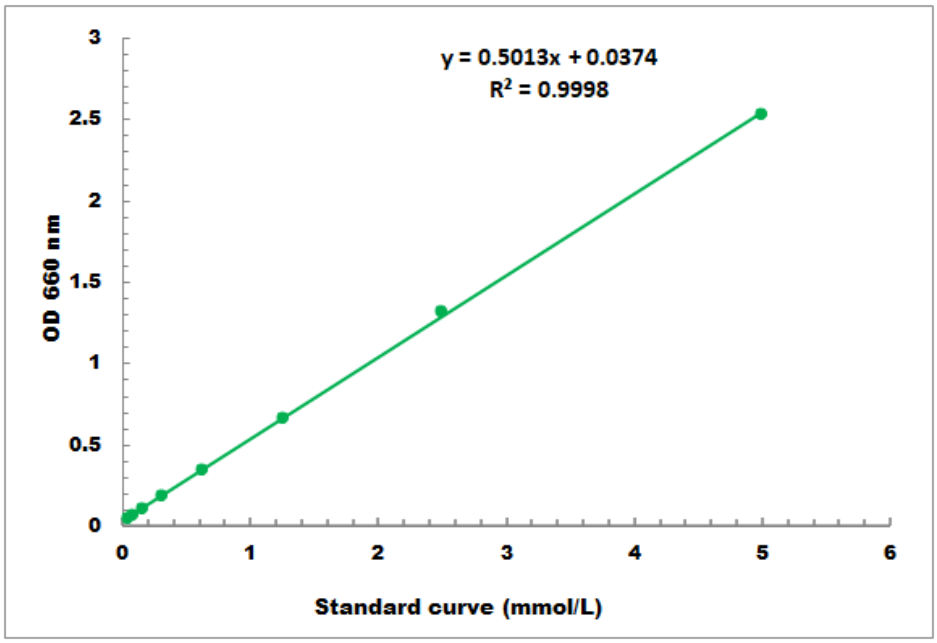
V_{Sample}: the volume of sample, 0.02 ml;

V_{Assay}: the volume of Assay buffer, 1 ml;

T: the reaction time, 30 minutes.

VII. TYPICAL DATA

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



Detection Range: 0.01 µmol/ml - 5 µmol/ml

VIII. TECHNICAL SUPPORT

For troubleshooting, information or assistance, please go online to www.cohesionbio.com or contact us at techsupport@cohesionbio.com

IX. NOTES