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# Ca<sup>++</sup>Mg<sup>++</sup>-ATPase Activity Assay Kit

**Note:** Take two or three different samples for prediction before test.

**Operation Equipment:** Spectrophotometer/Microplate reader

**Cat No:** AK0501 **Size:** 100T/48S

## **Components:**

Reagent I: Liquid 60 mL×1. Storage at 4°C. Reagent II: Liquid 4 mL×1. Storage at 4°C.

Reagent III: Powder $\times 2$ . Storage at -20°C . Dissolve thoroughly with 1 mL of distilled water before use. The

rest reagent can be kept at -20°C for one week.

Reagent IV: Liquid 2 mL×1. Storage at 4°C.

Reagent V: Powder×1. Storage at 4°C. Dissolve thoroughly with 3 mL of distilled water before use.

Reagent VI: Powder $\times 1$ . Storage at 4°C . Dissolve thoroughly with 5 mL of distilled water before use, can be kept at 4°C for one week.

Reagent VII: Powder $\times 1$ . Storage at 4°C . Dissolve thoroughly with 5 mL of distilled water before use, can be kept at 4°C for one week.

Reagent VIII: Liquid 5 mL×1. Storage at RT.

Standard solution: Liquid 1 mL×1. 10 µmol/mL standard phosphorus liquid, storage at 4°C.

**0.5**  $\mu$ mol/mL standard phosphorus working solution: Dilute the 10  $\mu$ mol/mL standard 20 times with distilled water to 0.5  $\mu$ mol/mL standard. For example: add 1.9 mL of distilled water to 0.1 mL of standard, mix thoroughly.

# **Phosphorus fixing reagent:**

Prepare reagents for determining phosphorus content: make solution as the volume ratio of  $H_2O$ : Reagent VII: Reagent VIII =2:1:1:1, which should be light yellow. It shows lose efficacy if color is changed, phosphorus pollution if color is change to blue. Prepare the reagent when it will be use.

**Note:** It is better to use new beakers, glass rods and glass pipettes or disposable plastic ware when making reagent to avoid phosphorus pollution.

## **Product Description:**

Ca<sup>++</sup>Mg<sup>++</sup>-ATPase is widely distributed in plants, animals, microorganisms and cells, which catalyzes the hydrolysis of ATP to form ADP and inorganic phosphorus.

Ca<sup>++</sup>Mg<sup>++</sup>-ATPase decomposes ATP to produce ADP and inorganic phosphorus. The activity of ATPase can be detected by measuring the amount of inorganic phosphorus.

# Reagents and Equipment Required but Not Provided:

Spectrophotometer/microplate reader, desk centrifuge, adjustable pipette, water bath, micro glass cuvette/96 well flat-bottom plate, mortar/homogenizer, ice and distilled water.

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## **Procedure:**

# Sample preparation:

# 1. Bacteria or cells:

Collecting bacteria or cells into a centrifuge tube, centrifugation and discard supernatant. Suggest add 1 mL of Reagent I to 5 million of bacteria or cells. Use ultrasonic to splitting bacteria and cells (placed on ice, ultrasonic power 20%, working time 3 seconds, interval 10 seconds, repeat for 30 times). Centrifuge at  $8000 \times g$  for 10 minutes at 4°C and take the supernatant on ice before test.

# 2. Tissue:

Add 1 mL of Reagent I into 0.1 g of tissue, fully grinding on ice. Centrifuge at 8000 ×g for 10 minutes at 4°C and take the supernatant on ice before test.

3. Serum: Detect directly.

# **II. Determination:**

- Preheat spectrophotometer/microplate reader for 30 minutes, adjust the wavelength to 660 nm, set the counter to zero with distilled water.
- Add the following reagents to EP tube:

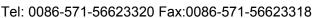
Reagent (µL)	Control tube (C)	Test tube (T)				
Reagent I	65	45				
Reagent II	40	40				
Reagent III	20	20				
Reagent IV		20				
Sample		100				
Mix thoroughly, then place the reaction solution in a 37°C (mammal) or 25°C (other species) water						
bath for 10 minutes						
Reagent V	25	25				
Sample	100					
Mix thoroughly, centrifuge at 4000 ×g for 10 minutes at room temperature, take the supernatant.						

Determination of phosphorus content, add the following reagents to 1.5 mL EP tube: 3.

Reagent (μL)	Blank tube (B)	Standard tube (S)	Control tube (C)	Test tube (T)
0.5 μmol/mL		20		
standard		20		
phosphorus liquid				
Supernatant			20	20
Distilled water	20			
Reagents for	200	200	200	200
determining				
phosphorus content				

Mix thoroughly, then place the mix solution in a 40°C water bath for 10 minutes. Cooling to room temperature and detect the absorbance at 660 nm. The blank tube and standard tube just need one or two tubes.

# **III.Calculation:**



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#### 1. Serum:

Unit definition: One unit of enzyme activity is defined as the amount of enzyme catalyzes the decompose of ATP to produce 1 µmol of inorganic phosphorus per hour every milliliter of serum.

$$Ca^{++}Mg^{++}$$
-ATPase  $(U/mL)$ = $Cs\times[\Delta A(T)-\Delta A(C)]\div[\Delta A(S)-\Delta A(B)]\times Vrv\div s\div T$ 

$$=7.5\times[\Delta A(T)-\Delta A(C)]\div[\Delta A(S)-\Delta A(B)]$$

## 2. Tissue, bacteria or cells

# (1) Protein concentration:

Unit definition: One unit of enzyme activity is defined as the amount of enzyme catalyzes the decompose of ATP to produce 1 µmol of inorganic phosphorus per hour every milligram of tissue protein.

$$Ca^{++}Mg^{++}$$
-ATPase (U/mgprot)= $Cs\times[\Delta A(T)-\Delta A(C)]\div[\Delta A(S)-\Delta A(B)]\times Vrv\div(Vs\times Cpr)\div T$ 

=7.5×
$$[\Delta A(T)-\Delta A(C)]$$
÷ $[\Delta A(S)-\Delta A(B)]$ ÷Cpr

# (2) Sample weight:

Unit definition: One unit of enzyme activity is defined as the amount of enzyme catalyzes the decompose of ATP to produce 1 µmol of inorganic phosphorus per hour every milligram of tissue.

$$Ca^{++}Mg^{++}-ATPase~(U/g~weight) = Cs \times [\Delta A(T)-\Delta A(C)] \div [\Delta A(S)-\Delta A(B)] \times Vrv \div (Vs \div V1 \times W) \div T$$

=7.5×[
$$\Delta$$
A(T)- $\Delta$ A(C)]÷[ $\Delta$ A(S)- $\Delta$ A(B)]÷W

# (3) bacteria or cells

Unit definition: One unit of enzyme activity is defined as the amount of enzyme catalyzes the decompose of ATP to produce 1 µmol of inorganic phosphorus per hour every 10000 cells or bacteria.

$$Ca^{++}Mg^{++}$$
-ATPase (U/104cell )= $Cs \times [\Delta A(T) - \Delta A(C)] \div [\Delta A(S) - \Delta A(B)] \times Vrv \div (Vs \div V1 \times 500) \div T$ 

$$=0.015\times[\Delta A(T)-\Delta A(C)]\div[\Delta A(S)-\Delta A(B)]$$

Cs: Concentrate of standard tube, 0.5 µmol/mL;

Vrv: Total reaction volume, 0.25 mL;

Vs: Sample volume, 0.1 mL;

Cpr: Sample protein concentration (mg/mL);

T: Reaction time (min), 1/6 hour;

W: Sample weight(g);

VI: Volume of Reagent I, 1 mL;

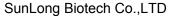
500: The amount of bacteria or cell, 5 million.

## Note:

- 1. This kit can detect 48 tubes of Ca<sup>++</sup>Mg<sup>++</sup>-ATPase samples in 100 tubes for each sample need one tube as control.
- 2. This method has the characteristics of trace, sensitive and rapid. The test tubes used for determination are phosphate-free strictly. Avoiding phosphorus pollution is the key to the success of detection.

## **Experimental example:**

1. Take 0. 1g of pancreas and add 1 mL of Reagent I for ice bath homogenization. After centrifugation at 4°C for 10 min, the supernatant is put on the ice and operated according to the determination steps. Using





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96 well plate,  $\Delta A_T = 0.535 - 0.238 = 0.297$ ,  $\Delta A_S = 0.280 - 0.043 = 0.237$ 

Ca++Mg++- ATPase activity (U/g mass) =  $7.5 \times \Delta A_T \div \Delta A_S \div W = 93.99$  U/g mass.

2. Take 0. 1g of willow and add 1 mL of Reagent I for ice bath homogenization. After centrifugation at 4°C for 10 min, the supernatant is put on ice and operated according to the determination steps. The  $\Delta A_T$ =0. 105-0.099 = 0.006, and the  $\Delta A_S$ = 0.280-0.043 = 0.237

Ca + + Mg + + - ATPase activity (U/g mass) =  $7.5 \times \Delta AT \div \Delta AS \div W = 1.90 \text{ U/g mass}$ .

# **Recent Product Citations:**

[1] Yupu Jing, Hongli An, Shanjing Zhang, et al. Protein kinase C mediates juvenile hormone-dependent phosphorylation of Na<sup>+</sup>. Journal of Biological Chemistry. November 2018;(IF4. 106)

## References:

[1] Datiles M J, Johnson E A, McCarty R E. Inhibition of the ATPase activity of the catalytic portion of ATP synthases by cationic amphiphiles[J]. Biochimica et Biophysica Acta (BBA)-Bioenergetics, 2008, 1777(4): 362-368.

## **Related Products:**

AK0602/AK0601 Na+K+——ATPase Activity Assay Kit

AK0309/AK0561 ATP Activity Assay Kit