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$Ca^{++}Mg^{++}$-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℃ .

Reagent II: Liquid 4 mL×1. Storage at 4℃ .

Reagent III: Powder×2. Storage at -20℃ . Dissolve thoroughly with 1 mL of distilled water before use. The rest reagent can be kept at -20℃ for one week.

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

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

Reagent VI: Powder×1. Storage at 4℃ . Dissolve thoroughly with 5 mL of distilled water before use, can be kept at 4℃ for one week.

Reagent VII: Powder×1. Storage at 4℃ . Dissolve thoroughly with 5 mL of distilled water before use, can be kept at 4℃ 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℃ .

0.5 μmol/mL standard phosphorus working solution: Dilute the 10 μmol/mL standard 20 times with distilled water to 0.5 μmol/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 VI: 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:

$Ca^{++}Mg^{++}$-ATPase is widely distributed in plants, animals, microorganisms and cells, which catalyzes the hydrolysis of ATP to form ADP and inorganic phosphorus.

$Ca^{++}Mg^{++}$-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.

Procedure:

I. 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 ×g for 10 minutes at 4℃ 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℃ and take the supernatant on ice before test.

3. Serum: Detect directly.

II. Determination:

1. Preheat spectrophotometer/microplate reader for 30 minutes, adjust the wavelength to 660 nm, set the counter to zero with distilled water.

2. 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℃ (mammal) or 25℃ (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.		

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

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

Mix thoroughly, then place the mix solution in a 40℃ 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:

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×[ΔA(T)-ΔA(C)]÷[ΔA(S)-ΔA(B)]×Vrv÷s÷T

=7.5×[ΔA(T)-ΔA(C)]÷[ΔA(S)-ΔA(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×[ΔA(T)-ΔA(C)]÷[ΔA(S)-ΔA(B)]×Vrv÷(Vs×Cpr)÷T

=7.5×[ΔA(T)-ΔA(C)]÷[ΔA(S)-ΔA(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×[ΔA(T)-ΔA(C)]÷[ΔA(S)-ΔA(B)]×Vrv÷(Vs÷V1×W)÷T

=7.5×[ΔA(T)-ΔA(C)]÷[ΔA(S)-ΔA(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/10^4cell)=Cs×[ΔA(T)-ΔA(C)]÷[ΔA(S)-ΔA(B)]×Vrv÷(Vs÷V1×500)÷T

=0.015×[ΔA(T)-ΔA(C)]÷[ΔA(S)-ΔA(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);

Vl: Volume of Reagent I, 1 mL;

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

Note:

1. This kit can detect 48 tubes of $Ca^{++}Mg^{++}$-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℃ for 10 min, the supernatant is put on the ice and operated according to the determination steps. Using

96 well plate, ΔA_T = 0.535-0.238 = 0.297, ΔA_S = 0.280-0.043 = 0.237

Ca++Mg++- ATPase activity (U/g mass) = 7.5 × $\Delta A_T \div \Delta A_S \div$ 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℃ for 10 min, the supernatant is put on ice and operated according to the determination steps. The ΔA_T=0. 105-0.099 = 0.006, and the ΔA_S= 0.280-0.043 = 0.237

Ca + + Mg + + - ATPase activity (U/g mass) = 7.5×ΔAT ÷ ΔAS ÷ W = 1.90 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^+. 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