$Ca^{++}Mg^{++}$-ATPase Activity Assay Kit

Note: It is necessary to predict 2-3 large difference samples before the formal determination.

Operation Equipment: Spectrophotometer/Microplate reader

Cat No: BC0965

Size: 100T/48S

Product Composition: Before use, please carefully check whether the volume of the reagent is consistent with the volume in the bottle. If you have any questions, please contact Solarbio staff in time.

Reagent name	Size	Preservation Condition
Reagent I	Liquid 60 mL×1	2-8℃
Reagent II	Liquid 4 mL×1	2-8℃
Reagent III	Powder×2	-20℃
Reagent IV	Liquid 2 mL×1	2-8℃
Reagent V	Liquid 3 mL×1	2-8℃
Reagent VI	Powder×1	2-8℃
Reagent VII	Powder×1	2-8℃
Reagent VIII	Liquid 5 mL×1	RT
Standard solution	Liquid 1 mL×1	2-8℃

Solution Preparation:

1. Reagent III: Add 1 mL distilled water to one Reagent III before use. It could be stored at -20℃ for one week after dispensing to avoid repeated freezing and thawing.
2. Reagent VI: Dissolve with 5 mL of distilled water before use. The reagent can be stored at 2-8℃ for two weeks.
3. Reagent VII: Dissolve with 5 mL of distilled water before use. The reagent can be stored at 2-8℃ for two weeks.
4. Standard solution: 10 μmol/mL standard phosphorus liquid. 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.
5. Phosphorus fixing reagent: Before use, prepare according to the sample size in the ratio of distilled water: Reagent VI: Reagent VII: reagent VIII =2: 1: 1: 1. The prepared reagent should be light yellow. It shows lose efficacy if color is changed, phosphorus pollution if color is change to blue.

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.

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Reagents and Equipment Required but Not Provided:

Spectrophotometer/microplate reader, desk centrifuge, water bath/constant temperature incubator, adjustable pipette, micro glass cuvette/96 well flat-bottom plate, mortar/homogenizer/cell ultrasonic crusher, 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, the spectrophotometer needs to be zeroed 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, record as A_T, A_C, A_S, A_B. $\Delta A_T=A_T-A_C$, $\Delta A_S=A_S-A_B$. The blank and standard tubes only need to be measured 1-2 times.

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 activity (U/mL) $=Cs\times\Delta A_T\div\Delta A_S\times Vrv\div Vs\div T\times F$

$=7.5\times\Delta A_T\div\Delta A_S\times F$

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 activity (U/mgprot)$=Cs\times\Delta A_T\div\Delta A_S\times Vrv\div(Vs\times Cpr)\div T\times F$

$=7.5\times\Delta A_T\div\Delta A_S\div Cpr\times F$

(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 activity (U/g weight) $=Cs\times\Delta A_T\div\Delta A_S\times Vrv\div(Vs\div V1\times W)\div T\times F$

$=7.5\times\Delta A_T\div\Delta A_S\div W\times F$

(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 activity (U/10^4cell)$=Cs\times\Delta A_T\div\Delta A_S\times Vrv\div(Vs\div V1\times N)\div T\times F$

$=7.5\times\Delta A_T\div\Delta A_S\div N\times F$

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;

N: The amount of bacteria or cell, count by 10^4;

F: Dilution factor.

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. ΔA_T= 0.535-0.238=0.297, ΔA_S=0.280-0.043=0.237. The activity is calculated according to the sample mass:

$Ca^{++}Mg^{++}$- 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. ΔA_T=0.105- 0.099=0.006, ΔA_S=0.280-0.043=0.237. The activity is calculated according to the sample mass:

Ca^{++} Mg^{++} - ATPase activity (U/g mass) = 7.5×$\Delta A_T \div \Delta A_S \div$ W = 1.90 U/g mass.

Recent Product Citations：

[1] Sun J, Qu H, Ali W, Chen Y, Wang T, Ma Y, Yuan Y, Gu J, Bian J, Liu Z, Zou H. Co-exposure to cadmium and microplastics promotes liver fibrosis through the hemichannels -ATP-P2X7 pathway. Chemosphere. 2023 Dec; 344:140372. doi: 10.1016/j.chemosphere.2023.140372. Epub 2023 Oct 4. PMID: 37802476.

[2] Chen HX, Wang XC, Hou HT, Wang J, Yang Q, Chen YL, Chen HZ, He GW. Lysine crotonylation of SERCA2a correlates to cardiac dysfunction and arrhythmia in Sirt1 cardiac-specific knockout mice. Int J Biol Macromol. 2023 Jul 1;242(Pt 4):125151. doi: 10.1016/j.ijbiomac.2023.125151. Epub 2023 Jun 1. PMID: 37270127.

[3] Li L, Qi Q, Zhang H, Dong Q, Iqbal A, Gui H, Kayoumu M, Song M, Zhang X, Wang X. Ameliorative Effects of Silicon against Salt Stress in Gossypium hirsutum L. Antioxidants (Basel). 2022 Aug 4;11(8):1520. doi: 10.3390/antiox11081520. PMID: 36009240; PMCID: PMC9404900.

[4] Wang L, Li A, Fang J, Wang Y, Chen L, Qiao L, Wang W. Enhanced Cell Wall and Cell Membrane Activity Promotes Heat Adaptation of Enterococcus faecium. Int J Mol Sci. 2023 Jul 23;24(14):11822. doi: 10.3390/ijms241411822. PMID: 37511581; PMCID: PMC10380804.

[5] Sun J, Yu F, Wang T, Bian J, Liu Z, Zou H. The role of DRP1- PINK1-Parkin-mediated mitophagy in early cadmium-induced liver damage. Toxicology. 2022 Jan 30; 466:153082. doi: 10.1016/j.tox.2021. 153082. Epub 2021 Dec 21. PMID: 34952138.

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.

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BC0060/BC0065	Na^+K^+-ATPase Activity Assay Kit
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