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

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

Operation Equipment: Spectrophotometer

Cat No: BC0960

Size: 50T/24S

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 30 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 15 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 15 mL of distilled water before use. The reagent can be stored at 2-8℃ for two weeks.
3. Reagent VII: Dissolve with 15 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, desk centrifuge, water bath/constant temperature incubator, adjustable pipette, 1 mL glass cuvette, 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. It is suggested that add 1mL of Reagent I to 5 million of bacteria or cells. Use ultrasonic to splitting bacteria and cells (placed on ice, ultrasonic power 200w, 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 testing.

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 testing.

3. Serum: Detect directly.

II. Determination:

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

2. Add the following reagents to EP tube:

Reagent (μL)	Control tube (C)	Test tube (T)
Reagent I	130	90
Reagent II	80	80
Reagent III	40	40
Reagent IV	-	40
Sample	-	200
Mix thoroughly, then place the reaction solution in a 37℃ (mammal) or 25℃ (other species) water bath for 10 minutes.		
Reagent V	50	50
Sample	200	-
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	-	100	-	-
Supernatant	-	-	100	100
Distilled water	100	-	-	-
Reagents for determining phosphorus content	1000	1000	1000	1000

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/mg prot) $=Cs\times\Delta A_T\div\Delta A_S\times Vrv\div(Vs\times Cpr)\div T\times F$

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

(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 gram 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 number:

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, 0.5 mL;

Vs: Sample volume, 0.2 mL;

Cpr: Sample protein concentration (mg/mL);

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

W: Sample weight(g);

V1: Volume of reagent I, 1 mL;

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

F: dilution factor.

Note:

1. This kit can detect 24 tubes of $Ca^{++}Mg^{++}$-ATPase samples in 50 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= 0.916-0.389=0.527, ΔA_S=0.398-0.004=0.394. The activity is calculated according to the sample mass:

$Ca^{++}Mg^{++}$- ATPase activity (U/g mass) = $7.5 \times \Delta A_T \div \Delta A_S \div W$ = 100.32 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=0.137- 0.124=0.013, ΔA_S=0.398-0.004=0.394. The activity is calculated according to the sample mass:

$Ca^{++}Mg^{++}$ - ATPase activity (U/g mass) = $7.5 \times \Delta A_T \div \Delta A_S \div W$ = 2.47 U/g mass.

Recent Product Citations:

[1] Chen T, Zhao MX, Tang XY, Wei WX, Wen X, Zhou SZ, Ma BH, Zou YD, Zhang N, Mi JD, Wang Y, Liao XD, Wu YB. The tigecycline resistance gene tetX has an expensive fitness cost based on increased outer membrane permeability and metabolic burden in Escherichia coli. J Hazard Mater. 2023 Sep 15;458:131889. doi: 10.1016/j.jhazmat.2023.131889. Epub 2023 Jun 19. PMID: 37348375.

[2] Sun J, Chen Y, Wang T, Ali W, Ma Y, Yuan Y, Gu J, Bian J, Liu Z, Zou H. Cadmium promotes nonalcoholic fatty liver disease by inhibiting intercellular mitochondrial transfer. Cell Mol Biol Lett. 2023 Oct 27;28(1):87. doi: 10.1186/s11658-023-00498-x. PMID: 37884867; PMCID: PMC10604759.

[3] Zhang W, Chen R, Xu K, Guo H, Li C, Sun X. Protective effect of Xinmai'an tablets via mediation of the AMPK/SIRT1/PGC-1 signaling pathway on myocardial ischemia-reperfusion injury in rats. Phytomedicine. 2023 Nov;120:155034. doi: 10.1016/j.phymed.2023.155034. Epub 2023 Aug 16. PMID: 37611465.

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[5] Zhang W, Li B, Lv Y, Wei S, Zhang S, Hu Y. Transcriptomic analysis shows the antifungal mechanism of honokiol against Aspergillus flavus. Int J Food Microbiol. 2023 Jan 2;384:109972. doi: 10.1016/j.ijfoodmicro.2022.109972. Epub 2022 Oct 12. PMID: 36279642.

References:

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[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:

BC0060/BC0065	Na^+K^+ -ATPase Activity Assay Kit
BC0300/BC0305	ATP Activity Assay Kit

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