Phosphotransacetylase (PTA) 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: BC5465

Size:100T/48S

Components:

Extract solution: Liquid 60mL×1. Store at 2-8℃.

Reagent I: Liquid 15mL×1. Store at 2-8℃.

Reagent II: Liquid 1.2mL×1. Store at 2-8℃.

Reagent III: Liquid 1.3mL×1. Store at 2-8℃.

Reagent IV: Powder×2. Store at -20℃. Add 0.6mL distilled water to each Reagent IV and mix well before use. It can be stored at -20℃ for two weeks after dispensing to avoid repeated freezing and thawing. (one reagent IV dissolved can do 60S, in order to extend the use of the kit, this product gives 1 more powder).

Product Description:

Phosphotransacetylase (PTA, EC 2.3.1.8) is one of the key enzymes related to acetate metabolism. Acetate kinase (ACK) and PTA catalyze acetic acid to generate acetyl coenzyme A, which is an important substance in the metabolism of carbohydrate, fat and protein.

PTA catalyzes acetyl coenzyme A and phosphate to produce acetyl phosphate and coenzyme A, following the transformation from DTNB to yellow TNB. TNB has a characteristic absorption peak at 412 nm, and PTA activity is calculated by measuring the rate of increase in absorbance at 412nm.

Reagents and Equipment Required but Not Provided:

Spectrophotometer/Microplate reader, water-bath, desk centrifuge, balance, transferpettor, micro glass cuvette/96-well flat-bottom plate, mortar/homogenizer/cell ultrasonic crusher, ice and distilled water.

Procedure:

I. Sample preparation

1. **Tissue:** According to the proportion of tissue weight (g): Extract solution volume (mL) of 1:5-10 to extract. It is suggested that 0.1 g of tissue with 1 mL of Extract solution and fully homogenized on ice bath. Centrifuge at 12000rpm for 10 minutes at 4℃ to remove insoluble materials and take the supernatant on ice before testing.
2. **Bacteria or cells:** Collect bacteria or cells into the centrifuge tube, after centrifugation discard supernatant. According to the proportion of bacteria or cells number (10^4): Extract solution volume (mL) of 500-1000-1 to extract. It is suggested that add 1 mL of Extract solution to 5 million of bacteria or cells. Use ultrasonication to split bacteria or cells (place on ice, ultrasonic power 200W, working

time 3 seconds, interval 7 seconds, repeat for 5 minutes). Centrifuge at 12000rpm for 10 minutes at 4℃ to remove insoluble materials and take the supernatant on ice for testing.

II. Determination

1. Preheat spectrophotometer/microplate reader for 30 min, adjust the wavelength to 412 nm and set spectrophotometer counter to zero with distilled water.
2. Keep Reagent I at 25℃ for 10 min.
3. Add reagents in micro glass cuvette/96-well flat-bottom plate as the following:

Reagent (μL)	Test tube	Contrast tube
Reagent I	120	130
Reagent II	10	10
Reagent III	10	10
Sample	50	50
Reagent IV	10	-

Add the above reagents into the micro glass cuvette/96-well plate in order, mix well, and record the initial absorbance A1 at 412 nm for 15 seconds. After colorimetry, quickly put the cuvette together with the reaction solution into a 25℃ water bath and react accurately for 2 minutes (if the enzyme marker is equipped with the function of controlling the temperature, adjust the temperature to 25℃); remove the cuvette and dry it quickly, and record the absorbance A2 at 412 nm for 2 minutes and 15 seconds,Calculate $\Delta A_T=A_{T2}-A_{T1}$, $\Delta A_C=A_{C2}-A_{C1}$, $\Delta A=\Delta A_T-\Delta A_C$. Each test tube should be provided with one contrast tube.

III. Phosphotransacetylase activity calculation:

A. micro glass cuvette:

1. Protein concentration:

Unit definition: One unit of enzyme activity is defined as the amount of enzymes catalyzes production of 1 nmol TNB in the reaction system per minute at 25℃ every mg protein.

PTA activity (U/mg prot) =ΔA÷(ε×d) ×Vrv÷(Cpr×Vs)÷T=147.059×ΔA ÷Cpr

2. Sample weight:

Unit definition: One unit of enzyme activity is defined as the amount of enzymes catalyzes production of 1 nmol TNB in the reaction system per minute at 25℃ every g sample.

PTA activity (U/g weight) = ΔA÷(ε×d) ×Vrv÷(W×Vs÷Ve)÷T=147.059×ΔA÷W

3. Bacteria or cell amount:

Unit definition: One unit of enzyme activity is defined as the amount of enzymes catalyzes production of 1 nmol TNB in the reaction system per minute at 25℃ every 10^6 bacteria or cells.

PTA activity (U/10^6 cell) = ΔA÷(ε×d) ×Vrv÷(N×Vs÷Ve)÷T=147.059×ΔA÷N

ε: extinction coefficient, 13.6×10^{-3} mL/nmol/cm;

d: Light path of cuvette, 1cm;

Vrv: Total reaction volume, 0.2mL;

Cpr: Crude enzyme protein concentration, mg/mL;

Vs: Crude enzyme volume, 0.05 mL;

T: Reaction time, 2 min;

W: Sample weight, g;

Ve: Extract solution volume, 1mL;

N: Bacteria or cell amount, 10^6 for one unit.

B. 96-well flat-bottom plate:

Modify d-1 cm in the above formula to d-0.6 cm (light path of the cuvette) for calculation.

Note:

1. Samples and regents should be placed on ice to avoid denaturation.
2. It is recommended to measure and count time respectively by two people to ensure the accuracy.
3. According to samples number, working solution could be prepared with Reagent I, Reagent II and Reagent III. But it is not recommended to measure more than one sample simultaneously.
4. If ΔA_T is low, it is recommended to increase the sample size before determination. If ΔA_T>0.6(micro glass cuvette) or 0.4(96-well flat-bottom plate), it is recommended to dilute the sample with Extract solution before determination. And modify the calculation formula.

Experimental example:

1. Take 0.1056g mature pear fruit, add 1 mL of Extract solution, grind the homogenate with ice bath. Then operate according to the determination steps, calculate $\Delta A_T=A_{T2}-A_{T1}$=0.1013-0.0620=0.0393. $\Delta A_C=A_{C2}-A_{C1}$=0.0571-0.0558=0.0013, $\Delta A=\Delta A_T-\Delta A_C$=0.0393-0.0013=0.0380. The result is calculated according to the sample weight:
   PTA activity (U/g weight)= 147.059×ΔA÷W =52.919 U/g weight
2. Take 5.76×10^6 cells, add 0.8 mL of Extract solution, grind the homogenate with ice bath. Then operate according to the determination steps, calculate $\Delta A_T=A_{T2}-A_{T1}$=0.2411-0.1402=0.1009. $\Delta A_C=A_{C2}-A_{C1}$=0.1775-0.1037=0.0738, $\Delta A=\Delta A_T-\Delta A_C$=0.1009-0.0738=0.0271. The result is calculated according to cells numbers:
   PTA activity (U/10^6 cell)= 0.0271÷（$13.6\times10^{-3}\times1$）×0.2÷（5.76×0.05÷0.8）÷2=0.554 U/10^6 cell

References：

[1] Michael M, Karin B, Wolfgang S, et al. Isolation and properties of acetate kinase- and phosphotransacetylase- negative mutants of Thermoanaerobacter thermohydrosulfuricus [J]. Microbiology, 1995, 141(11): 2891-2896.

[2] Miyake M, Kataoka K, Shirai M, et al. Control of poly-beta-hydroxybutyrate synthase mediated by acetyl phosphate in cyanobacteria[J]. Journal of Bacteriology, 1997, 179(16): 009-5013.

[3] Bock AK, Glasemacher J, Schmidt R, et al. Purification and characterization of two extremely thermostable enzymes, phosphate acetyltransferase and acetate kinase, from the

hyperthermophilic eubacterium Thermotoga maritima[J]. Journal of Bacteriology, 1999, 181(6): 1861-1867.

Related Products：

BC6020/BC6025	Acetyl CoA carboxylase(ACC) Activity Assay Kit（Enzymatic method）
BC0980/BC0985	Acetyl Coenzyme A Content Assay Kit
BC3170/BC3175	Acetate Kinase (ACK) Activity Assay Kit

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