Phosphotransacetylase (PTA) Activity Assay Kit

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

Operation Equipment: Spectrophotometer

Cat No: BC5460

Size:50T/24S

Components:

Extract solution: Liquid 30mL×1. Store at 2-8℃.

Reagent I: Liquid 40mL×1. Store at 2-8℃.

Reagent II: Liquid 3mL×1. Store at 2-8℃.

Reagent III: Liquid 3mL×1. Store at 2-8℃.

Reagent IV: Powder×2. Store at -20℃. Before use, take one of reagent IV and add 1.5 mL of distilled water to fully dissolve it. It can be stored at -20℃ for two weeks after dispensing to avoid repeated freezing and thawing. (one reagent IV dissolved can do 30S, 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, water-bath, desk centrifuge, balance, mortar/homogenizer/cell ultrasonic crusher, transferpettor, 1 mL glass cuvette, 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 for 30 min, adjust the wavelength to 412 nm and set the counter to zero with distilled water.
2. Keep Reagent I at 25℃ for 10 min.
3. Add reagents in 1 mL glass cuvette as the following:

Reagent (μL)	Test tube	Contrast tube
Reagent I	600	650
Reagent II	50	50
Reagent III	50	50
Sample	250	250
Reagent IV	50	-

Mix thoroughly. Record the initial absorbance A1 at the wavelength of 412 nm for 15s and incubate for 2min at 25℃. Record the absorbance A2 at the wavelength of 412 nm for 2min15s. 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. Calculation:

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) $=\Delta A\div(\varepsilon\times d)\times Vrv\div(Cpr\times Vs)\div T=147.059\times\Delta A\div Cpr$

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) $=\Delta A\div(\varepsilon\times d)\times Vrv\div(W\times Vs\div Ve)\div T=147.059\times\Delta A\div W$

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) $=\Delta A\div(\varepsilon\times d)\times Vrv\div(N\times Vs\div Ve)\div T=147.059\times\Delta A\div N$

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

d: Light path of cuvette, 1cm;

Vrv: Total reaction volume, 1mL;

Cpr: Crude enzyme protein concentration, mg/mL;

Vs: Crude enzyme volume, 0.25 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.

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 $\Delta A_T>0.6$, it is recommended to dilute the sample with Extract solution before determination. And modify the calculation formula.

Experimental example:

1. Take 0.1099g mature peach fruit, add 1 mL of Extract solution, grind the homogenate with ice bath. Then operate according to the determination steps, calculate $\Delta A_T=A_{T2}-A_{T1}=0.105-0.069=0.036$. $\Delta A_C=A_{C2}-A_{C1}=0.062-0.060=0.002$, $\Delta A=\Delta A_T-\Delta A_C=0.036-0.002=0.034$. The result is calculated according to the sample weight:
   PTA activity (U/g weight)= $147.059\times\Delta A\div W$ =45.496 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 $\Delta A_T=A_{T2}-A_{T1}=0.188-0.116=0.072$. $\Delta A_C=A_{C2}-A_{C1}=0.131-0.087=0.044$, $\Delta A=\Delta A_T-\Delta A_C=0.072-0.044=0.028$. The result is calculated according to cells numbers:
   PTA activity (U/10^6 cell)= $0.028\div(13.6\times10^{-3}\times1)\times1\div(5.76\times0.25\div0.8)\div2=0.572$ 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