Acetate kinase (ACK) activity Assay Kit

Note: Take two or three different samples for prediction before test.

Operation Equipment: Spectrophotometer/ Microplate Reader

Cat No: BC3175

Size:100T/96S

Components:

Extract solution: Liquid 100 mL×1. Store at 4°C. Add reagent IX to the extract solution before use and the dissolved reagent store at 4°C.

Reagent I: Liquid 15 mL×1. Store at 4°C.

Reagent II: Powder×1. Store at 4°C. Dissolve with 1 mL of distilled water before use. The unused reagents store at 4°C.

Reagent III: Powder×2. Store at -20°C. Dissolve with 1 mL of distilled water before use. The unused reagents store at -20°C.

Reagent IV: Powder×1. Store at -20°C. Dissolve with 2 mL of distilled water before use. The unused reagents store at -20°C.

Reagent V: Powder×1. Store at -20°C. Dissolve with 0.5 mL of distilled water before use. The unused reagents store at 4°C.

Reagent VI: Liquid 48 μL×1. Store at 4℃. Prepare Reagent VI and distilled water according to the sample size at the ratio of 43:457 (V: V) before use. Prepared when the solution will be used.

Reagent VII: Liquid 55 μL×1. Store at -20°C. Prepare Reagent VII and distilled water according to the sample size at the ratio of 1:9 (V: V) before use. Prepared when the solution will be used.

Reagent VIII: Powder×1. Store at 4°C. Dissolve with 5 mL of distilled water before use. The unused reagents store at 4°C.

Reagent IX: Powder×1. Store at 4°C.

Working solution: Take 0.06 mL of reagent II, 0.2 mL of reagent III, 0.13 mL of reagent IV, 0.04 mL of reagent V, 0.04 mL of reagent VI, 0.04 mL of reagent VII, 0.2 mL of reagent VIII and mix them well, keep on ice. Working solution can also be mixed in accordance with the proportion of the above, keep on ice.

Product Description:

Acetate kinase (ACK) is widely present in organisms, which catalyzes acetic acid and ATP to acetophosphate and ADP. ACK is the key enzyme in carbon and energy metabolism of bacteria, especially plays a central role in the methane anabolic metabolism of archaea.

Principle: ACK catalyzes acetic acid and ATP to acetophosphate and ADP. Pyruvate kinase catalyzes ADP and phosphoenolpyruvic acid(PEP) to ATP and pyruvic acid. Lactic dehydrogenase (LDH) catalyzes pyruvic acid and NADH to lactic acid and NAD^+. In this kit, the activity of ACK is determined by the decrease rate of NADH at 340 nm.

Reagents and Equipment Required but Not Provided:

Spectrophotometer/microplate reader, desk centrifuge, adjustable transferpettor, water bath, micro quartz cuvette/96 well flat-bottom UV plate, mortar/homogenizer, ice, distilled water.

Procedure:

I. Sample preparation:

1. Bacteria or cells

Collecting bacteria or cells into the centrifuge tube, after centrifugation discard supernatant. Suggest add 1 mL of Extract reagent to 5 million of bacteria or cells. Use ultrasonication to splitting bacteria and cells (placed on ice, ultrasonic power 20%, working time 3 seconds, interval 10 seconds, repeat for 30 times). Centrifuge at 15000 ×g for 20 minutes at 4℃ to remove insoluble materials and take the supernatant on ice before testing.

2. Tissue

Add 1 mL of Extract reagent to 0.1 g of tissue, and fully homogenized on ice bath. Centrifuge at 15000×g for 20 minutes at 4℃ to remove insoluble materials, and take the supernatant on ice before testing.

3. Serum: detect directly.

II. Determination procedure:

1. Preheat spectrophotometer or microplate reader for 30 minutes, adjust the wavelength to 340 nm, set zero with distilled water.
2. Preheat Reagent I for 15 minutes at 37℃(mammal) or 25℃ (other species).
3. Add the following reagents

Reagent (μL)	Test tube (T)	Blank tube (B)
Distilled water	-	20
Reagent I	110	110
Working solution	70	70
Sample	20	-

Add the above reagents to micro quartz cuvette/96 well UV plate in order, timing after add working solution, mix thoroughly. Detect the absorbance at 340 nm at the time of 20 seconds record as A_{T1} or A_{B1}. Then place dishes with the reaction solution in a 37℃ water bath for 3 minutes. Take it out and wipe it clean, immediately measure the absorbance at the time of 200 seconds which record as A_{T2} or A_{B2}. $\Delta A_T = A_{T2} - A_{T1}$, $\Delta A_B = A_{B2} - A_{B1}$, $\Delta A = \Delta A_T - \Delta A_B$. The blank tube need only be tested one or two times.

III. Calculation:

1. Protein concentration:

Unit definition: One unit of enzyme activity is defined as the amount of enzyme that generates 1 nmol of NADH per minute every milligram of protein.

ACK (U/mg prot) $=[\Delta A \div (\varepsilon \times d) \times 10^9 \times Vrv] \div (Vs \times Cpr) \div T = 536 \times \Delta A \div Cpr$

2. Sample weight:

Unit definition: One unit of enzyme activity is defined as the amount of enzyme that generates 1

nmol of NADH per minute every gram of tissue.

ACK (U/g weight) = $[\Delta A \div (\varepsilon \times d) \times 10^9 \times Vrv] \div (W \div Ve \times Vs) \div T = 536 \times \Delta A \div W$

3. Bacteria or cultured cells

Unit definition: One unit of enzyme activity is defined as the amount of enzyme that generates 1 nmol of NADH per minute every 10 thousand cells or bacteria.

ACK (U/10^4 cell) $=[\Delta A \div (\varepsilon \times d) \times 10^9 \times Vrv] \div (Vs \div Ve \times 500) \div T = 1.072 \times \Delta A$

4. Serum(plasma):

Unit definition: One unit of enzyme activity is defined as the amount of enzyme that generates 1 nmol of NADH per minute every milliliter of serum or plasma.

ACK (U/mL) $=[\Delta A \times Vrv \div (\varepsilon \times d) \times 10^9] \div Vs \div T = 536 \times \Delta A$

Vrv: Total reaction volume, 2×10^{-4} L;

ε: NADH molar extinction coefficient, 6.22×10^3 L/mol/cm;

d: Light path of cuvette, 1 cm;

Vs: Sample volume, 0.02 mL;

Ve: Extract volume, 1 mL;

T: Reaction time, 3 minutes;

Cpr: Sample protein concentration (mg/mL);

W: Sample weight(g);

500: 5 million cells or bacteria;

10^9: 1 mol $=10^9$ nmol.

A. 96 well UV plate

The d-1cm in the above formula is changed to d-0.6cm (optical diameter of 96 well flat-bottom plate) for calculation.

Note:

1. In the process of preparing for testing, sample and all of reagents should be keep on ice to prevent enzyme deactivation.
2. Keep 37℃ or 25℃ of the react solution in cuvette, add 37℃ or 25℃ water to a beaker, put this beaker in 37℃ or 25℃ water bath and put the cuvette in this beaker.
3. If A>1, please dilute the sample to appropriate concentration, multiply dilute times in the formula. If A<0.01, prolong the enzymatic reaction time and pay attention to calculation formula changes.

Experimental Example:

1. Take 0.1g kidney tissue sample, add 1 mL extract solution for homogenate grinding, take the supernatant and operate according to the determination steps. Measure with micro quartz plate and calculate $\Delta A_T = A_{T1} - A_{T2}$ = 1.7666-1.0488 = 0.7178, $\Delta A_B = A_{B1} - A_{B2}$ = 1.3902-1.3553 = 0.0349, $\Delta A = \Delta A_T - \Delta A_B$ = 0.7178-0.0349 = 0.6829

ACK (U/g mass) = 536 × ΔA÷W = 536 × 0.6829÷0.1 = 3660.344 U/g mass.

2. Take 5 million E. coli and add 1 mL extract solution to ultrasonic crushing, centrifugation, take the supernatant and operate according to the determination steps. Measure with micro quartz plate and calculate $\Delta A_T = A_{T1} - A_{T2} = 1.3965 - 1.3503 = 0.0462$, $\Delta A_B = A_{B1} - A_{B2} = 1.3902 - 1.3553 = 0.0349$, $\Delta A = \Delta A_T - \Delta A_B = 0.0462 - 0.0349 = 0.0113$

ACK（U/10^4 cell）$=1.072\times\Delta A=1.072\times0.0113=0.0121136$ U/10^4 cell

References：

[1] Mukhopadhyay S, Hasson M S, Sanders D A. A continuous assay of acetate kinase activity: Measurement of inorganic phosphate release generated by hydroxylaminolysis of acetyl phosphate[J]. Bioorganic chemistry, 2008, 36(2): 65-69.

Related Products：

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