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CheKine™ Micro Acetokinase (ACK) Activity Assay Kit

Cat #: KTB1127 Size: 48 T/96 T

	Micro Acetokinase (ACK) Activity Assay Kit		
REF	**Cat #:** KTB1127	LOT	**Lot #:** Refer to product label
	Applicable samples: Animal and Plant Tissues, Cells or Bacteria, Plasma, Serum or other Liquid samples		
	Storage: Stored at -20℃ for 6 months, protected from light		

Assay Principle

Acetokinase (ACK) is primarily found in microorganisms and functions as a key enzyme in bacterial carbon metabolism and energy metabolism, playing a central role particularly in the methanogenesis pathway of archaea. ACK catalyzes the conversion of acetate and ATP into acetyl phosphate and ADP. Subsequently, pyruvate kinase catalyzes the formation of ATP and pyruvate from ADP and PEP. Lactate dehydrogenase then catalyzes the reaction that converts pyruvate and NADH into lactate and NAD+. By measuring the rate of NADH oxidation to NAD^+ at a wavelength of 340 nm, one can determine the activity of ACK.

Materials Supplied and Storage Conditions

Kit components	Size		Storage conditions
	48 T	96 T	
Extraction Buffer	50 mL	100 mL	4℃
Reagent Ⅰ	10 mL	30 mL	4℃
Reagent Ⅱ	1	1×2	-20℃, protected from light
Reagent Ⅲ	23 μL	46 μL	4℃, protected from light

Materials Required but Not Supplied

- Microplate reader or ultraviolet spectrophotometer capable of measuring absorbance at 340 nm
- 96-well UV plate or microquartz cuvette, precision pipettes, disposable pipette tips
- Water bath, ice maker, centrifuge
- Deionized water
- Homogenizer (for tissue samples)

Reagent Preparation

Extraction Buffer: Ready to use as supplied. Equilibrate to room temperature before use. Store at 4℃.

Reagent Ⅰ: Ready to use as supplied. Equilibrate to room temperature before use. Store at 4℃.

Working Reagent Ⅱ: Prepare before use, take one bottle of Reagent Ⅱ, add 10 mL of Reagent Ⅰ and 18 μL of Reagent III, thoroughly mix and dissolve. Prepare immediately before use. The mixture can be stored at -20°C, protected from light, for 1 month.

Reagent III: Ready to use as supplied. Equilibrate to room temperature before use. Store at 4℃, protected from light.

Sample Preparation

Note: It is recommended to use fresh samples. If the experiment is not conducted immediately, the samples can be stored at -80℃ for 1 month. The temperature and time of thawing should be controlled during the determination. When thawing at room temperature, the sample should be thawed within 4 h.

1. Tissues: Weigh 0.1 g tissue , add 1 mL Extraction Buffer, homogenize on ice. Centrifuge at 15,000 g for 10 min at 4℃. Take the supernatant and place it on the ice for testing.
2. Cells or Bacteria: Collect 5×10^6 cells or bacteria into the centrifuge tube, wash cells or bacteria with cold PBS, discard the supernatant after centrifugation; add 1 mL Extraction Buffer to ultrasonically disrupt the cells or bacteria (power 200 W, ultrasonic 3 s, interval 10 s, repeat 30 times). Centrifuge at 15,000 g for 10 min at 4℃. Use supernatant for assay, and place it on ice to be tested.
3. Serum, Plasma and other Liquid Samples: Direct detection. If the solution has turbidity, centrifuge and take the supernatant for measurement.

Note: If the protein concentration of the sample is need to determined, it is recommended to use Abbkine catalog number: KTD3001 Protein Quantification Kit (BCA Assay) to measure the protein concentration of the sample.

Assay Procedure

1. Preheat the microplate reader or ultraviolet spectrophotometer for more than 30 min, and adjust the wavelength to 340 nm, ultraviolet spectrophotometer was returned to zero with deionized water.
2. According to experimental requirements, take out an appropriate portion of the pre-prepared Reagent Ⅱ and place it in a water bath set at 37°C (for mammalian species) or 25°C (for other species) for 5 min. Ensure that the reagent is freshly prepared immediately prior to use.
3. Sample measurement. (The following operations are operated in the 96-well UV plate or microquartz cuvette)

Regent	Test well (μL)
Sample	20
Working Reagent Ⅱ	180
Mix well, record the absorbance values of 10 s and 190 s at 340 nm, mark as A_1 and A_2, and calculate $\Delta A=A_1-A_2$.	

Note: In order to guarantee the accuracy of experimental results, need to do a pre-experiment with 2-3 samples. If the ΔA is less than 0.05, the sample size can be appropriately increased or the reaction time can be appropriately extended to 10 min or 20 min for detection. If the ΔA is greater than 1.0, the sample can be appropriately diluted with Extraction Buffer or reduce the sample quality used for extraction, the calculated result multiplied by the dilution factor.

Data Analysis

Note: We provide you with calculation formulae, including the derivation process and final formula. The two are exactly equal. It is suggested that the concise calculation formula in bold is final formula.

A. 96-well UV plates calculation formula

(1) Calculated by protein concentration:

Unit definition: One enzyme activity unit defines as 1 nmol NADH consumed by 1 mg tissue proteins per min.

ACK (U/mg prot)=$[\Delta A\times V_{Total}\div(\varepsilon\times d)\times10^9]\div(V_{Sample}\times Cpr)\div T$**$=1{,}072\times\Delta A\div Cpr$**

(2) Calculation according to the weight of the sample:

Unit definition: One enzyme activity unit defines as 1 nmol NADH consumed by 1 g tissue per min

ACK (U/g fresh weight)=[ΔA×V_{Total}÷(ε×d)×10^9]÷(V_{Sample}×W÷$V_{Sample\ Total}$)÷T**=1,072×ΔA÷W**

(3) Calculation according to the number of bacteria or cell:

Unit definition: One enzyme activity unit defines as 1 nmol NADH consumed by 10^4 bacteria or cells per min

ACK (U/g 10^4)=[ΔA×V_{Total}÷(ε×d)×10^9]÷(500×V_{Sample}÷$V_{Sample\ Total}$)÷T**=2.144×ΔA**

(4) Calculation according to the volume of liquid:

Unit definition: One enzyme activity unit defines as 1 nmol NADH produced by 1 mL liquid per min

ACK (U/mL)=[ΔA×V_{Total}÷(ε×d)×10^9]÷V_{Sample}÷T**=1,072×ΔA**

Where: ε: NADH molar extinction coefficient, 6.22×10^3 L/mol/cm; d: 96-well UV plate diameter, 0.5 cm; V_{Total}: the total volume of the reaction system, 0.2 mL=2×10^{-4} L; V_{Sample}: the volume of the sample in the reaction system, 0.02 mL; $V_{Sample\ Total}$: The volume of Extraction Buffer added, 1 mL; Cpr: protein concentration (mg/mL); W: sample weight, g; T: reaction time, 3 min; 500: Total number of bacteria or cells, 5×10^6.

B. Microquartz cuvette calculation formula

The optical diameter d:0.5 cm in the above calculation formula can be adjusted to d:1 cm for calculation.

Precautions

1. During the measurement process, samples and all reagents should be kept on ice to prevent denaturation and inactivation.
2. The reaction solution's temperature must be maintained at either 37℃ or 25℃. To achieve this during spectrophotometric measurements, a small beaker can be filled with an appropriate amount of deionized water preheated to 37℃ or 25℃. This beaker is then placed inside a water bath set at the corresponding temperature of 37℃ or 25℃. Throughout the reaction process, the cuvette containing the reaction mixture should be positioned within this heated beaker.

Typical Data

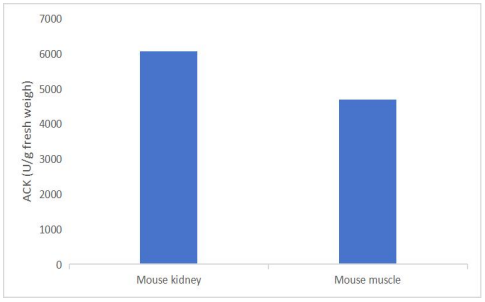

Figure 1. ACK activity in Mouse kidney and Mouse muscle was detected with this kit

Recommended Products

Catalog No.	Product Name
KTB1015	CheKine™ Micro α-glucosidase(α-GC) Activity Assay Kit
KTB1121	CheKine™ Micro Pyruvate Acid (PA) Assay Kit

Disclaimer

The reagent is only used in the field of scientific research, not suitable for clinical diagnosis or other purposes.