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CheKine™ Micro Pyruvate Kinase (PK) Assay Kit

Cat #: KTB1120 Size: 48 T/96 T

	Micro Pyruvate Kinase (PK) Assay Kit		
REF	**Cat #:** KTB1120	LOT	**Lot #:** Refer to product label
	Applicable samples: Serum, Plasma, Animal and Plant Tissues, Cells, Bacteria		
	Storage: Stored at -20℃ for 12 months		

Assay Principle

Pyruvate Kinase (PK, EC 2.7.1.40) is widely present in animals, plants, microorganisms and cultured cells, and catalyzes the last step reaction in the glycolysis process. It is one of the main rate-limiting enzymes in the glycolysis process and the key to ATP production. It is important to measure PK activity. CheKine™ Micro Pyruvate Kinase (PK) Assay Kit provides a simple method for detecting PK activity in a variety of biological samples such as Serum, Plasma, Animal Tissues, Cells and Bacteria. PK catalyzes phosphoenolpyruvate and ADP reaction into ATP and pyruvate, and lactate dehydrogenase further catalyzes the production of lactic acid and NAD^+ by NADH and pyruvate. The rate of NADH decline at 340 nm can reflect PK activity.

Materials Supplied and Storage Conditions

Kit components	Size		Storage conditions
	48 T	96 T	
Extraction Buffer	50 mL	100 mL	4℃
Assay Buffer	10 mL	20 mL	4℃
Substrate Mix	1	1	-20℃
LDH	1	1	4℃

Materials Required but Not Supplied

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

Reagent Preparation

Extraction Buffer: Ready to use as supplied. Equilibrate to room temperature before use. Store at 4℃.
Assay Buffer: Ready to use as supplied. Equilibrate to room temperature before use. Store at 4℃.
Substrate Mix Working Reagent: Prepared before use. Add 8.5 mL Assay Buffer and 0.5 mL Deionized Water to the 48 T Substrate Mix bottle to fully dissolve, add 17 mL Assay Buffer and 1 mL Deionized Water to the 96 T Substrate Mix bottle to fully dissolve. Stored aliquots at -20℃ for 6 months, avoid repeated freezing and thawing.
LDH Working Reagent: Prepared before use. Add 0.5 mL deionized water to LDH in 48 T kit and mix well, add 1 mL deionized water to LDH in 96 T kit. After dilution, it can be stored at 4℃ for 1 month and kept on ice for use.

Sample Preparation

Note: Fresh samples are recommended, If not assayed immediately, samples can be stored at -80℃ for one month.
1. Animal Tissues: Weigh 0.1 g tissue, add 1 mL Extraction Buffer and homogenize on ice. Centrifuge at 8,000 g for 10 min at 4℃. Use supernatant for assay, and place it on ice to be tested.
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 5 min on ice (power 20% or 200 W, ultrasonic 3 s, interval 7 s, repeat 30 times). Centrifuge at 8,000 g for 10 min at 4℃. Use supernatant for assay, and place it on ice to be tested.
3. Serum, Plasma or other Liquid samples: Tested directly.
Note: If the protein concentration of the sample is need to determined, it is recommended to use Abbkine Cat #: 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. Substrate Mix Working Reagent place at 37°C (mammal) or 25°C (other species) incubation for 5 min.
3. Add 10 μL sample, 10 μL LDH Working Reagent and 180 μL Substrate Mix Working Reagent to the 96-well UV plate or microquartz cuvette, mix quickly.
4. Measure the absorbance value at 340 nm with a microplate reader, record 20 s absorbance value as A_1 and the absorbance value at 2 min 20 s as 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 ΔA_{Test} is less than 0.001, increase the sample quantity appropriately. If ΔA_{Test} is greater than 1.0, the sample can be appropriately diluted with Extraction Buffer, the calculated result multiplied by the dilution factor, or decrease the sample quantity appropriately.

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 as below
1. Calculation of PK activity in serum (plasma)
Active unit definition: 1 nmol NADH consumed per min in 1 mL Serum (Plasma) reaction system is defined as a unit of enzyme activity.
$PK\ (U/mL)=[\Delta A\times V_{Total}\div(\varepsilon\times d)\times10^9]\div V_{Sample}\div T$**$=3,215\times\Delta A$**
2. Calculation of PK activity in tissues, bacteria or cells

(1) Calculated by protein concentration

Active unit definition: 1 nmol NADH consumed per min in 1mg tissue protein reaction system is defined as a unit of enzyme activity.

PK (U/mg prot)=[ΔA×V_{Total}÷(ε×d)×10^9]÷(Cpr×V_{Sample})÷T**=3,215×ΔA÷Cpr**

(2) Calculated by sample fresh weight

Active unit definition: 1 nmol NADH consumed per min in 1 g tissue reaction system is defined as a unit of enzyme activity.

PK (U/g fresh weight)=[ΔA×V_{Total}÷(ε×d)×10^9]÷(V_{Sample}÷$V_{Total\ Sample}$×W)÷T**=3,215×ΔA÷W**

(3) Calculated by bacteria or cell number

Active unit definition: 1 nmol NADH consumed per min in 10^4 bacteria or cells reaction system is defined as a unit of enzyme activity.

PK (U/10^4)=[ΔA×V_{Total}÷(ε×d)×10^9]÷(V_{Sample}÷$V_{Total\ Sample}$×500)÷T=3215×ΔA÷500**=6.431×ΔA**

Where: V_{Total}: total reaction volume, 2×10^{-4} L; ε: NADH molar extinction coefficien, 6.22×10^3 L/mol/cm; d: 96-well plate diameter, 0.5 cm; 10^9: 1 mol=1×10^9 nmol; V_{Sample}: sample volume added, 0.01 mL; $V_{Total\ Sample}$: extract solution volume added, 1 mL; T: reaction time, 2 min; Cpr; sample protein concentration, mg/mL; W: sample weight, g; 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.

Recommended Products

Catalog No.	Product Name
KTB1110	CheKine™ Micro Lactate Dehydrogenase (LDH) Activity Assay Kit
KTB1300	CheKine™ Micro Glucose Assay Kit
KTB1100	CheKine™ Micro Lactate Assay Kit
KTB1310	CheKine™ Micro Glucose oxidase (GOD) Activity Assay Kit

Disclaimer

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