CheKine™ Micro Pyruvate Carboxylate (PC) Activity Assay Kit

Cat #: KTB1119 Size: 48 T/48 S 96 T/96 S

	Micro Pyruvate Carboxylase (PC) Activity Assay Kit		
REF	**Cat #:** KTB1119	LOT	**Lot #:** Refer to product label
	Applicable samples: Animal Tissues, Cells		
	Storage: Stored at -20℃ for 6 months, protected from light		

Assay Principle

Pyruvate carboxylase (PC) is widely present in mitochondria of animals, molds and yeast, but is not found in plants and most bacteria. PC is the main postreaction for oxaloacetate, and is the first rate limiting enzyme in the gluconeogenesis process. CheKine™ Micro Pyruvate Carboxylase (PC) Activity Assay Kit can be used to detect biological samples such as animal tissues, cells. In the kit, PC irreversibly catalyzes pyruvate, ATP, CO_2 and water to oxaloacetate, ADP and Pi, malic dehydrogenase further catalyzes the formation of malic acid and NAD^+ from acetoacetic acid and NADH. The enzyme activity of PC can be reflected by detecting the oxidation rate of NADH at 340 nm.

Materials Supplied and Storage Conditions

Kit components	Size		Storage conditions
	48 T	96 T	
Extraction Buffer	60 mL	60×2 mL	4℃
Reagent Ⅰ	10.8 mL	21.6 mL	4℃, protected from light
Reagent Ⅱ	Powder×1 vial	Powder×1 vial	4℃, protected from light
Reagent Ⅲ	Powder×1 vial	Powder×1 vial	-20℃, protected from light
Reagent Ⅳ	Powder×1 vial	Powder×1 vial	-20℃, protected from light

Note: Before formal testing, it is recommended to select 2-3 samples with large expected differences for pre-experiment.

Materials Required but Not Supplied

- Microplate reader or visible spectrophotometer capable of measuring absorbance at 340 nm
- 96-well UV plate or microglass cuvette, precision pipettes, disposable pipette tips, 1.5 mL EP tube
- Water bath, cryogenic centrifuge
- Deionized water
- Homogenizer or mortar

Reagent Preparation

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

Note: Extraction Buffer is toxic and has a pungent odor, so it is recommended to experiment in a fume hood.

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

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

Reagent III: Ready to use as supplied; Equilibrate to room temperature before use; Store at -20℃, protected from light.

Reagent IV: Prepared before use. 48 T add 0.6 mL deionized water, 96 T add 1.2 mL deionized water, fully dissolve. The remaining reagent can also be stored at -20℃ for 1 month and protected from light after aliquoting to avoid repeated freezing and thawing.

Working Reagent: Prepared before use. Dissolve Reagent II and Reagent III with Reagent Ⅰ, transfer dissolved Reagent II and Reagent III to Reagent Ⅰ, fully dissolve. The remaining reagent can also be stored at -20℃ for 1 month and protected from light after aliquoting to avoid repeated freezing and thawing.

Sample Preparation

Note: We recommend that you use fresh samples. If not assayed immediately, samples can be stored at -80℃ for one month.When measuring, the temperature and time of thawing should be controlled. When thawing at room temperature, the sample should be thawed within 4 h.

Separation of cytoplasmic and mitochondrial proteins from animal tissues and cells: Weigh 0.1 g tissue or collect 5×10^6 cells, add 1 mL Extraction Buffer, homogenize or mortar on ice. Centrifuge at 600 g for 5 min at 4℃. Abandon precipitation, and transfer the supernatant to another centrifugal tube;Centrifuge at 11,000 g for 5 min at 4℃. The supernatant is to remove the cytoplasmic protein of mitochondria, which can be used to determine the PC leakage from mitochondria (optional). The precipitation is mitochondria, adding 1 mL Extraction, ultrasonic fragmentation (ice bath, power 20% or 200 W, ultrasound 3 s, interval 10 s, repetition 30 times) for PC activity determination.

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 visible spectrophotometer for more than 30 min, and adjust the wavelength to 340 nm. Visible spectrophotometer was returned to zero with deionized water.
2. Preheat Working Reagent at 37℃ (mammals) or 25℃ (other species) for 5 min.
3. Sample measurement. (The following operations are operated in the 96-well UV plate or microglass cuvette)

Reagent	Test Well (μL)
Sample	10
Reagent IV	10
Working Reagent	180

4. After rapid mixing, the absorbance values A_1 at 10 s and A_2 at 130 s at 340 nm are recorded. Finally calculate $\Delta A=A_2-A_1$.

Note: In order to guarantee the accuracy of experimental results, need to do a pre-experiment with 2-3 samples. If ΔA is less than 0.01, increase the sample quantity or prolong the reaction time appropriately. If ΔA is greater than 0.5, 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

Calculation of the PC activity

(1) Calculated by sample protein concentration

Unit definition: One unit of enzyme is defined as the consumption of 1 nmol NADH per milligram protein.

$PC_{Supernatant}(U/mg\ prot)=[\Delta A_{Supernatant}\times V_{Total}\div(\varepsilon\times d)\times 10^9]\div(V_{Sample}\times Cpr)\div T$**$=3,216\times\Delta A_{Supernatant}\div Cpr$**

$PC_{Precipitate}(U/mg\ prot)=[\Delta A_{Precipitate}\times V_{Total}\div(\varepsilon\times d)\times 10^9]\div(V_{Sample}\times Cpr)\div T$**$=3,216\times\Delta A_{Precipitate}\div Cpr$**

Total $PC(U/mg\ prot)=PC_{Supernatant}+PC_{Precipitate}$**$=3,216\times\Delta A_{Supernatant}\div Cpr+3,216\times\Delta A_{Precipitate}\div Cpr$**

(2) Calculated by fresh weight of samples

Unit definition: One unit of enzyme is defined as the consumption of 1 nmol NADH per gram sample.

$PC_{Supernatant}(U/g\ fresh\ weight)=[\Delta A_{Supernatant}\times V_{Total}\div(\varepsilon\times d)\times 10^9]\div(W\times V_{Sample}\div V_{Total\ Sample})\div T$**$=3,216\times\Delta A_{Supernatant}\div W$**

$PC_{Precipitate}(U/g\ fresh\ weight)=[\Delta A_{Precipitate}\times V_{Total}\div(\varepsilon\times d)\times 10^9]\div(W\times V_{Sample}\div V_{Total\ Sample})\div T$**$=3,216\times\Delta A_{Precipitate}\div W$**

Total $PC(U/g\ fresh\ weight)=PC_{Supernatant}+PC_{Precipitate}$**$=3,216\times\Delta A_{Supernatant}\div W+3,216\times\Delta A_{Precipitate}\div W$**

(3) Calculated by number of cells or bacteria

Unit definition: One unit of enzyme is defined as the consumption of 1 nmol NADH per 10^4 cells.

$PC_{Supernatant}(U/g\ 10^4\ cell)=[\Delta A_{Supernatant}\times V_{Total}\div(\varepsilon\times d)\times 10^9]\div(n\times V_{Sample}\div V_{Total\ Sample})\div T$**$=3,216\times\Delta A_{Supernatant}\div n$**

$PC_{Precipitate}(U/g\ 10^4\ cell)=[\Delta A_{Precipitate}\times V_{Total}\div(\varepsilon\times d)\times 10^9]\div(n\times V_{Sample}\div V_{Total\ Sample})\div T$**$=3,216\times\Delta A_{Precipitate}\div n$**

Total $PC(U/g\ 10^4\ cell)=PC_{Supernatant}+PC_{Precipitate}$**$=3,216\times\Delta A_{Supernatant}\div n+3,216\times\Delta A_{Precipitate}\div n$**

$\Delta A_{Supernatant}$: OD value of supernatant; $\Delta A_{Precipitate}$: OD value of precipitate; ε: NADH molar extinction coefficient at 340 nm, 6.22×10^3 L/mol /cm; d: 96-well plate optical diameter, 0.5 cm; V_{Total}: Total reaction volume, 2×10^{-4} L; V_{Sample}: Sample volume added in the reaction system, 0.01 mL; $V_{Total\ Sample}$: The volume of added Extraction Buffer, 1 mL; Cpr; Sample protein concentration, mg/mL; W: Sample weight, g; T: Reaction time, 2 min; n: Total number of cells, calculated in units of ten thousand.

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.

Typical Data

The following data are for reference only. And the experimenters need to test the samples according to their own experiments.

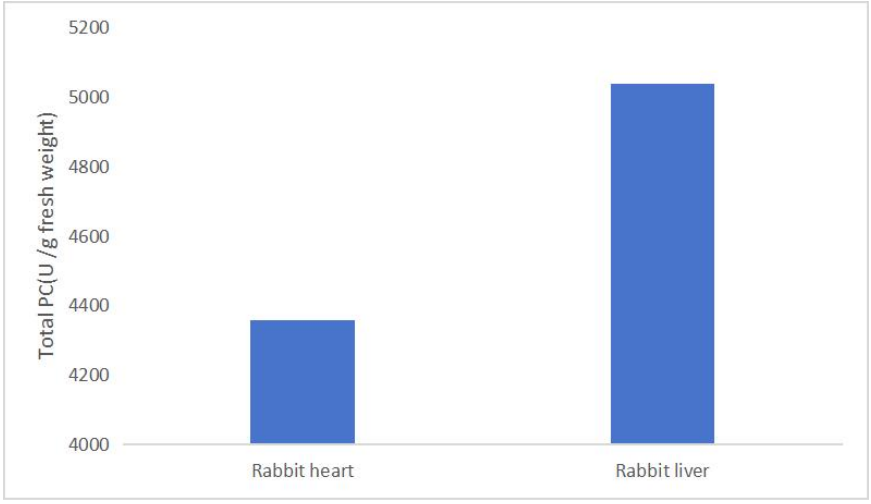

Figure 1. Determination of total PC activity in rabbit heart and liver by this kit.

Recommended Products

Catalog No.	Product Name

KTB1014	CheKine™ Micro Glucose-6-Phosphatase (G6P) Activity Assay Kit
KTB1126	CheKine™ Micro Phosphoenolpyruvate Carboxykinase (PEPCK) Activity Assay Kit

Disclaimer

The reagent is only used in the field of scientific research, not suitable for clinical diagnosis or other purposes. For your safety and health, please wear a lab coat and disposable gloves.