CheKine™ Micro Pyruvate Dehydrogenase (PDH) Activity Assay Kit

Cat #: KTB1270 Size: 48 T/48 S 96 T/96 S

	Micro Pyruvate Dehydrogenase (PDH) Activity Assay Kit		
REF	**Cat #:** KTB1270	LOT	**Lot #:** Refer to product label
	Applicable samples: Animal and Plant Tissues, Cells and Bacteria		
	Storage: Stored at -20℃ for 6 months, protected from light		

Assay Principle

Pyruvate dehydrogenase (PDH) exists widely in animals, plants, microorganisms and cultured cells. It is the rate-limiting enzyme of pyruvate dehydrogenase complex (PDHC) that catalyzes the decarboxylation of pyruvate to produce hydroxyethyl-TPP, and connects glycolysis with the tricarboxylic acid cycle. CheKine™ Micro Pyruvate Dehydrogenase (PDH) Activity Assay Kit provides a simple, convenient and rapid PDH activity detection method, which contains all necessary reagents and is suitable for testing animal, plant tissues, cell and bacteria samples. The detection principle is that PDH can catalyze pyruvate dehydrogenation, and reduce 2,6-Dichloroindophenol (2,6-DCPIP), the changes of 2,6-DCPIP could be detected by reading the absorption at 605 nm. The enzyme activity of PHD could be obtained by calculating the reduction rate of 2,6-DCPIP.

Materials Supplied and Storage Conditions

Kit components	Size		Storage conditions
	48 T	96 T	
Extraction Buffer Ⅰ	60 mL	60 mL×2	4℃
Extraction Buffer Ⅱ	0.75 mL	1.5 mL	-20℃, protected from light
Reagent Ⅰ	11.4 mL	22.8 mL	4℃
Reagent Ⅱ	Powder×1 vial	Powder×1 vial	4℃, protected from light
Reagent Ⅲ	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 605 nm
- Ice maker, refrigerated centrifuge
- Water bath
- 96-well plate or microglass cuvette

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- Precision pipettes, disposable pipette tips
- Deionized water
- Homogenizer (for tissue samples)

Reagent Preparation

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

Extraction Buffer Ⅱ: Ready to use as supplied. Equilibrate to room temperature before use. Store at -20℃, protected from light.

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

Working Solution: Prepare before use, Reagent Ⅱ and Reagent Ⅲ powder are all transferred to Reagent Ⅰ, fully mix and dissolve.The remaining Working Solution can also be stored at -20℃ and protected from light for 1 month after aliquoting to avoid repeated freezing and thawing.

Note: Extraction Buffer Ⅱ and Reagent Ⅱ have certain toxicity. Please take protective measures when operating.

Sample Preparation

Note: Fresh samples are recommended, If not assayed immediately, samples can be stored at -80℃ for one month. Processed samples must be assayed immediately. All samples and reagents should be on ice to avoid denaturation and deactivation.

1. Animal or plant tissues: Weigh 0.1 g tissues, add 1 mL Extraction Buffer Ⅰ and 10 μL Extraction Buffer Ⅱ and homogenize on ice. Centrifuge at 11,000 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 Ⅰ and 10 μL Extraction Buffer Ⅱ 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 11,000 g for 10 min at 4℃. Use supernatant for assay, and place it on ice to be tested.

Assay Procedure

1. Preheat the microplate reader or visible spectrophotometer for more than 30 min, and adjust the wavelength to 605 nm, visible spectrophotometer was returned to zero with deionized water.
2. Incubate Working Solution for 10 min at 37℃ (mammalia) or 25℃ (other species).
3. Add 10 μL sample, 190 μL Working Solution in a 96-well plate or microglass cuvette. Mix well, record the absorbance values of 20 s and 5 min 20 s at 605 nm with a microplate reader, 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. ΔA should be between 0.01-0.3, If ΔA is less than 0.01, increase the sample quantity appropriately or the reaction time can be extended for 10-15 min. If ΔA is greater than 0.3, the sample can be appropriately diluted with Extraction Buffer Ⅰ, the calculated result multiplied by the dilution factor, or decrease the sample quantity appropriately. It is not suggested to test too many samples at the same time, because enzyme activity is calculated by the variation of absorbance value per unit time.

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 plates calculation formula as below

1. Calculated by fresh weight of samples:

Unit definition: Every gram of tissue consumes 1 nmol of 2,6-DCPIP per min in the reaction system, which is defined as an enzyme activity unit.

PDH activity (U/g fresh weight)=$[\Delta A\times V_{Total}\div(\varepsilon\times d)\times10^9]\div(V_{Sample}\div V_{Extraction}\times W)\div T$**=384.76×ΔA÷W**

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2. Calculated by cell and bacteria density:

Unit definition: Every 10,000 cells and bacteria consume 1 nmol of 2,6-DCPIP per min in the reaction system, which is defined as an enzyme activity unit.

PDH activity ($U/10^4$)=[$\Delta A \times V_{Total} \div (\varepsilon \times d) \times 10^9$]÷($V_{Sample} \div V_{Extraction} \times 500$)÷T=**0.77×ΔA**

Where: V_{Total}: total reaction volume, 2×10^{-4} L; ε: 2,6-DCPIP molar extinction coefficient, 21×10^3 mol/L/cm; d: diameter of 96-well microplate, 0.5 cm; V_{Sample}: sample volume added, 0.01 mL; T: reaction time, 5 min; $V_{Extraction}$: sample extract volume, 1.01 mL; W: sample weight, g; 500: total number of cells or bacteria, 5×10^6.

B. Microglass 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
KTB1023	CheKine™ Micro Citrate Synthase (CS) Activity Assay Kit
KTB1230	CheKine™ Micro Succinate Dehydrogenase (SDH) Activity Assay Kit
KTB1240	CheKine™ Micro α-Ketoglutarate Dehydrogenase (α-KGDH) Assay Kit
KTB1250	CheKine™ Micro Mitochondrial Isocitrate Dehydrogenase (ICDHm) 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.

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