EZMeta™ Pyruvate Dehydrogenase (PDH) Colorimetric Activity Kit

Cat #: D-AKC1270

Size: 48T / 96T

Storage: Stored at -20°C for 6 months, protected from light

Product Information

Applicable samples: Animal and Plant Tissues, Cells and Bacteria

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. EZMeta™ Pyruvate Dehydrogenase (PDH) Colorimetric Activity 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	50 mL	100 mL	4°C
Reagent Ⅰ	10 mL	20 mL	4°C
Reagent Ⅱ	0.75 mL	1.5 mL	4°C, protected from light

Reagent Ⅲ	9.5 mL	19 mL	4°C
Reagent Ⅳ	1	1	4°C, protected from light
Reagent Ⅴ	1	1	-20°C, protected from light

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

·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°C.

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

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

Working Solution: Prepare before use, Reagent Ⅴ and Reagent Ⅳ powder are all transferred to Reagent Ⅲ, fully mix and dissolve, the prepared Working Solution can be stored from light at 4°C for one week. If it needs to be stored for a long time, it can be stored from light at -20°C after sub packaging to avoid repeated freezing and thawing. Equilibrate to room temperature before use.

Note: Reagent Ⅱ and Reagent Ⅳ 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°C for one month. Processed samples must be assayed immediately. All samples and reagents should be on ice to avoid denaturation and deactivation.

Extraction of cytoplasmic protein and mitochondrial protein from cells, bacteria and tissue:

1. Weigh 0.1 g tissue or collect 5×10^6 cells or bacteria, add 1 mL Extraction Buffer and 10 µL Reagent Ⅱ, homogenize on ice. Centrifuge at 600 g for 5 min at 4°C. Collect the supernatant to a new centrifuge tube and discard the pellet.

2. Centrifuge the supernatant again at 11,000 g for 10 min at 4°C, and obtain the supernatant and precipitate respectively.

3. (Optional) The supernatant collected in step 2 is cytoplasmic extract, which can be used to determine PDH leaking from mitochondria.

4. Add 200 µL Reagent I and 2 µL Reagent Ⅱ to the precipitate collected in step 2, resuspend the precipitate sufficiently, and use it to detect the activity of PDH in the next step.

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°C (mammalia) or 25°C (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 1 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. If ΔA is greater than 0.3, the sample can be appropriately diluted with Reagent 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.

$PDH_{Supernatant}$ activity (U/g fresh weight)=$[\Delta A_{Supernatant}\times V_{Total}\div(\varepsilon\times d)\times 10^9]\div(V_{Sample}\div V_{Extraction}\times W)\div T$=**$1{,}923.8\times\Delta A_{Supernatant}\div W$**

PDH_{Pellet} activity (U/g fresh weight)=$[\Delta A_{Pellet}\times V_{Total}\div(\varepsilon\times d)\times 10^9]\div(V_{Sample}\div V_{Total\ Sample}\times W)\div T$=**$384.76\times\Delta A_{Pellet}\div W$**

Total PDH activity (U/g fresh weight)=$PDH_{Supernatant}$ activity+PDH_{Pellet} activity=**$1{,}923.8\times\Delta A_{Supernatant}\div W+384.76\times\Delta A_{Pellet}\div W$**

2. Calculated by cell or bacteria density:

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

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

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, 1 min; $\Delta A_{Supernatant}$: OD value of supernatant; $V_{Extraction}$: sample extract volume, 1.01 mL; W: sample weight, g; ΔA_{Pellet}: OD value of pellet; $V_{Total\ Sample}$: the volume of adding Reagent I and II, 0.202 mL; 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.

Disclaimer

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