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Pyruvate Dehydrogenase (PDH) Activity Assay Kit

Note: The reagents of this product have changed, please pay attention to and strictly follow the instructions.

Operation Equipment: Spectrophotometer/Microplate reader

Catalog Number: BC0385

Size: 100T/96S

Product Composition: Before use, please carefully check whether the volume of the reagent is consistent with the volume in the bottle. If you have any questions, please contact Solarbio staff in time.

Reagent name	Size	Preservation Condition
Reagent I	Liquid 110 mL×1	2-8°C
Reagent II	Liquid 0.6 mL×2	-20°C
Reagent III	Liquid 7.5 mL×2	2-8°C
Reagent IV	Liquid 13 mL×1	2-8°C
Reagent V	Powder×2	-20°C
Reagent VI	Liquid 1 mL×1	2-8°C
Reagent VII	Liquid 4 mL×1	2-8°C

Solution Preparation:

1. Reagent II: Volatile reagent, seal it as soon as possible after use, store at -20°C.

2. Working solution: Add 5.75 mL of Reagent IV, 0.45 mL of Reagent VI, 1.75 mL of Reagent VII and one Reagent V to the bottle of Reagent III (15.45mL, about 85T), fully dissolved. It can be stored at -20°C for four weeks after dispensing to avoid repeated freezing and thawing

Product Description:

PDH widely exist in animals, plants, microorganism and cultured cells, which is the rate-limiting enzyme of acetylformic acid oxidative and decarboxylate catalyzed by Pyruvate dehydrogenase complex (PDHC). The decarboxylation of acetylformic acid forms hydroxyethyl-TPP, links glycolysis to the three carboxylic acid cycle.

PDH catalyzes the dehydrogenation of acetylformic acid and reduct 2, 6-dichlorophenol indophenol (2,6-DCPIP), which makes the absorption of 605 nm decrease.

Reagents and Equipment Required but Not Provided:

Spectrophotometer/microplate reader, water bath, desk centrifuge, adjustable pipette, micro glass cuvette/96 well flat-bottom plate, mortar/homogenizer, ice and distilled water.

Procedure:

I. Sample preparation:

1. Tissue: Weigh tissue sample of 0.1 g and add 1 mL of Reagent I and 10 μL of Reagent II, homogenate with mortar/homogenizer on ice. Centrifuge at 11000 g for 10 minutes at 4°C to remove insoluble

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materials and take the supernatant on ice before testing.

2. Cells or bacteria: Collect 5 million bacteria or cells into a centrifuge tube, add 1 mL of Reagent I and 10 µL of Reagent II to ultrasonically break bacteria or cells (power 200W, ultrasonic 3s, 7s interval,

total time 5 min). Centrifuge at 11000 g for 10 minutes at 4°C to remove insoluble materials and take the supernatant on ice before testing.

3. Serum or other liquid: Direct determination.

II. Determination procedure:

1. Preheat the spectrophotometer/microplate reader 30 minutes, adjust wavelength to 605 nm, set spectrophotometer counter to zero with distilled water.

2. Each sample requires 180 µL of working solution. Take a certain amount of working solution according to the number of samples and it at 37°C(mammal) or 25°C(other species) for 10 minutes.

3. Blank tube: Add 10 µL of distilled water, and 180 µL of working solution to micro glass cuvette/96 well flat-bottom plate. Mix thoroughly and timing, measure the absorption at 605 nm at the 10s and 1 minute, recorded as A1 and A2 respectively, calculate ΔA_B=A1-A2. **Blank tube only need to be measured once or twice**.

4. Test tube: Add 10 µL of supernatant, and 180 µL of working solution to micro glass cuvette/96 well flat-bottom plate. Mix thoroughly and timing, measure the absorption at 605 nm at the 10s and 1 minute, recorded as A3 and A4 respectively, calculate ΔA_T=A3-A4, ΔA=ΔA_T-ΔA_B.

III. PDH Calculation:

A. Micro quartz cuvette

1) Protein concentration:

Unit definition: One unit of enzyme activity is defined as the amount of enzyme catalyzes the consumption of 1 nmol of 2,6-DCPIP per minute every milligram of protein.

PDH (U/mg prot) = $[\Delta A \times Vrv \div (\varepsilon \times d) \times 10^9] \div (Vs \times Cpr) \div T = 904.762 \times \Delta A \div Cpr$

2) Sample weight

Unit definition: One unit of enzyme activity is defined as the amount of enzyme catalyzes the consumption of 1 nmol of 2,6-DCPIP per minute every gram tissue.

PDH (U/g weight)= $[\Delta A \times Vrv \div (\varepsilon \times d) \times 10^9] \div (W \times Vs \div Vsv) \div T = 913.81 \times \Delta A \div W$

3) Bacteria or cell density

Unit definition: One unit of enzyme activity is defined as the amount of enzyme catalyzes the consumption of 1 nmol of 2,6-DCPIP per minute every 10000 cells or bacteria.

PDH (U/ 10^4 cell)=$[\Delta A \times Vrv \div (\varepsilon \times d) \times 10^9] \div (500 \times Vs \div Vsv) \div T = 1.828 \times \Delta A$

4) Sample volume

Unit definition: One unit of enzyme activity is defined as the amount of enzyme catalyzes the consumption of 1 nmol of 2,6-DCPIP per minute every 1 mL of liquid.

PDH (U/mL)=$[\Delta A \times Vrv \div (\varepsilon \times d) \times 10^9] \div Vs \div T = 904.762 \times \Delta A$

Vrv: Reaction total volume, 1.9×10^{-4} L;

ε: Molar extinction coefficient, 2.1×10^4 L/mol/cm;

d: Light path of cuvette, 1 cm;

Vs: The sample volume, 0.01 mL;

Vsv: The Reagent I and II volume, 1.01 mL;

T: Reaction time, 1 minute;

Cpr: Sample protein concentration, mg/mL;

W: Sample quality, g;

500: The total number of bacteria and cells, 5 million.

B. 96 well flat-bottom plate

1）Protein concentration:

Unit definition: One unit of enzyme activity is defined as the amount of enzyme catalyzes the consumption of 1 nmol of 2,6-DCPIP per minute every milligram of protein.

$$PDH(U/mg\ prot) = [\Delta A \times Vrv \div (\varepsilon \times d) \times 10^9] \div (Vs \times Cpr) \div T = 1809.524 \times \Delta A \div Cpr$$

2）Sample weight

Unit definition: One unit of enzyme activity is defined as the amount of enzyme catalyzes the consumption of 1 nmol of 2,6-DCPIP per minute every gram of tissue.

$$PDH(nmol/min\ /mg\ weight) = [\Delta A \times Vrv \div (\varepsilon \times d) \times 10^9] \div (W \times Vs \div Vsv) \div T = 1827.62 \times \Delta A \div W$$

3）Bacteria or cell density

Unit definition: One unit of enzyme activity is defined as the amount of enzyme catalyzes the consumption of 1 nmol of 2,6-DCPIP per minute every 10000 cells or bacteria.

$$PDH(nmol/min/10^4\ cell) = [\Delta A \times Vrv \div (\varepsilon \times d) \times 10^9] \div (500 \times Vs \div Vsv) \div T = 3.655 \times \Delta A$$

4) Sample volume

Unit definition: One unit of enzyme activity is defined as the amount of enzyme catalyzes the consumption of 1 nmol of 2,6-DCPIP per minute every 1 mL of liquid.

$$PDH\ (U/mL) = [\Delta A \times Vrv \div (\varepsilon \times d) \times 10^9] \div Vs \div T = 1809.524 \times \Delta A$$

Vrv: Reaction total volume, 1.9×10^{-4} L;

ε: Molar extinction coefficient, 2.1×10^4 L/mol/cm;

d: Light path of cuvette, 0.6 cm;

Vs: The sample volume, 0.01 mL;

Vsv: The reagent I and II volume, 1.01 mL;

T: Reaction time, 1 minute;

Cpr: Sample protein concentration, mg/mL;

W: Sample quality, g;

500: The total number of bacteria and cells, 5 million.

Note:

1. During the measurement process, all samples are placed on the ice and tested within 2 hours to avoid denaturation and inactivation.

2. The measured value of ΔA should in range of 0.01~ 0.25. If ΔA>0.25, the sample should be properly diluted.

3. Since Reagent I contains a certain concentration of protein (about 1mg/mL), it is necessary to subtract the protein content of Reagent I when determining the concentration of sample protein.

Experimental Examples：

1. Take 0.1 g of mouse liver, add 1 mL of Reagent I and 10 μL Reagent II, grind the homogenate with ice bath, centrifuge at 11000g and 4℃ for 10 min, take the supernatant and put it on ice, operate according to

the determination steps, and calculate the ΔA_T=A3-A4= 1.3106-1.1183=0.1923, ΔA_B=A1-A2 = 1.3325-1.3314=0.0011.

PDH activity (U/g mass) = 913.81 × (ΔA_T- ΔA_B) ÷ W = 1747 U/g mass.

Recent Product Citations：

[1] Peng S, Wang Y, Zhou Y, et al. Rare ginsenosides ameliorate lipid overload-induced myocardial insulin resistance via modulating metabolic flexibility[J]. Phytomedicine, 2019, 58: 152745.

References：

[1] Guitart M, Andreu A L, García-Arumi E, et al. FATP1 localizes to mitochondria and enhances pyruvate dehydrogenase activity in skeletal myotubes[J]. Mitochondrion, 2009, 9(4): 266-272.

Related Products：

BC0710/BC0715　　Acetaldehyde Dehydrogenase(ALDH) Activity Assay Kit

BC2150/BC2155　　Citric Acid(CA) Content Assay Kit

BC0950/BC0955　　Succinate Dehydrogenase(SDH) Activity Assay Kit