Pyruvate Dehydrogenase (PDH) Activity Assay Kit

Note: It is necessary to predict 2-3 large difference samples before the formal determination.

Operation Equipment: Spectrophotometer

Catalog Number: BC0380

Size: 50T/48S

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 60 mL×1	2-8℃
Reagent II	Liquid 0.6 mL×1	-20℃
Reagent III	Liquid 15 mL×2	2-8℃
Reagent IV	Liquid 25 mL×1	2-8℃
Reagent V	Powder×2	-20℃
Reagent VI	Liquid 2 mL×1	2-8℃
Reagent VII	Liquid 8 mL×1	2-8℃

Solution Preparation:

1. Reagent II: Volatile reagent, seal it as soon as possible after use.
2. Working solution: Add 11.5 mL of Reagent IV, 0.9 mL of Reagent VI, 3.5 mL of Reagent VII and one Reagent V to the bottle of Reagent III (30.9mL, about 34T), fully dissolved. It can be stored at -20℃ for four weeks after dispensing to avoid repeated freezing and thawing

Product Description:

PDH is 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, water bath, desk centrifuge, adjustable pipette, mortar/homogenizer, 1 mL glass cuvette, 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℃ to remove insoluble 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℃ 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 30 minutes, adjust wavelength to 605 nm, set zero with distilled water.
2. Each sample requires 900 μL of working solution. Take a certain amount of working solution according to the number of samples plus one and it at 37℃(mammal) or 25℃(other species) for 10 minutes.
3. Blank tube: Add 50 μL of distilled water, and 900 μL of working solution to 1 mL quartz cuvette. Mix thoroughly and timing, measure the absorption at 605 nm at 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 50 μL of supernatant, and 900 μL of working solution to 1 mL quartz cuvette. Mix thoroughly and timing, measure the absorption at 605 nm at 10s and 1 minute, recorded as A3 and A4 respectively, calculate ΔA_T=A3-A4. $\Delta A=\Delta A_T-\Delta A_B$.

III. PDH Calculation:

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) = $[\Delta A\times Vrv\div(\varepsilon\times d)\times10^9]\div(Vs\times Cpr)\div T=904.762\times\Delta A\div Cpr$

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(nmol/min/mg weight)= $[\Delta A\times Vrv\div(\varepsilon\times d)\times10^9]\div(W\times Vs\div Vsv)\div T=913.81\times\Delta A\div W$

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(nmol/min/10^4 cell)=$[\Delta A\times Vrv\div(\varepsilon\times d)\times10^9]\div(500\times Vs\div Vsv)\div T=1.828\times\Delta A$

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)=$[\Delta A\times Vrv\div(\varepsilon\times d)\times10^9]\div Vs\div T=904.762\times\Delta A$

Vrv: Reaction total volume, 9.5×10^{-4} L;
ε: Molar extinction coefficient, 2.1×10^4 L/mol/cm;
d: Light path of cuvette, 1 cm;
Vs: The sample volume,0.05 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; If the ΔA < 0.01, it is necessary to increase the sample size and re-determine, and pay attention to the simultaneous modification of the calculation formula.
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 lung, 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 =1.226-1.015=0.211, ΔA_B=A1-A2 =1.442-1.439= 0.003.

PDH activity (U/g mass) = 913.81 × (ΔA_T- ΔA_B) ÷ W = 1900.72 U/g mass.

2. Take 0.1 g of Echinochloa crusgalli, 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=1.391-1.379=0.012, ΔA_B=A1-A2 = 0.

PDH activity (U/g mass) = 913.81 × (ΔAT- ΔAB) ÷ W = 109.66 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