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Pyruvate Dehydrogenase(PDH) Activity Assay Kit

Note: Take two or three different samples for prediction before test.

Operation Equipment: Spectrophotometer/Microplate reader

Cat No: AK0553

Size: 100T/96S

Components:

Reagent I: Liquid 110 mL×1. Storage at 4℃.

Reagent Ⅱ: Liquid 1 mL×1. Storage at -20℃. Protect from light.

Reagent Ⅲ: Liquid 20 mL×1. Storage at 4℃.

Reagent Ⅳ: Powder×1. Storage at 4℃.

Reagent V: Powder×1. Storage at -20℃.

Reagent VI: Powder×1. Storage at 4℃. Add 1 mL of distilled water before use.

Reagent VII: Powder×1. Storage at 4℃.

Working solution: Add Reagent Ⅳ, Reagent V, Reagent VII and 0.5 mL of Reagent VI to Reagent Ⅲ, fully dissolved.

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:

Weigh tissue sample of 0.1 g or collect cells sample of 5 million 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.

II. Determination procedure:

1. Preheat the spectrophotometer/microplate reader 30 minutes, adjust wavelength to 605 nm, set 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℃(mammal) or 25℃(other species) for 5 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 initial time and 1 minute, recorded as A1 and A2 respectively, calculate ΔA_B=A1-A2.

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 initial time 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:

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

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)= [ΔA×Vrv÷(ε×d)×10^9]÷(W×Vs÷Vsv)÷T=1827.62×ΔA÷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)=[ΔA×Vrv÷(ε×d)×10^9]÷(500×Vs÷Vsv)÷T=3.655×ΔA

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, 0.5 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 determination, all samples are placed on ice 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 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 = 0.701-0.615 = 0.086, ΔA_B=A1-A2 = 0.9-0.898 =
0.002.
PDH activity (U/g mass) = 1827.62 × (ΔA_T- ΔA_B) ÷ W = 1535.2 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 = 0.835-0.813 = 0.022, ΔA_B=A1-A2 = 0.898-0.897 = 0.001.
PDH activity (U/g mass) = 1827.62 × (ΔAT- ΔAB) ÷ W = 383.8 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:

AK0282/AK0281	Acetaldehyde Dehydrogenase(ALDH) Activity Assay Kit
AK0400/AK0399	Citric Acid(CA) Content Assay Kit
AK0504/AK0503	Succinate Dehydrogenase(SDH) Activity Assay Kit