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Pyruvate Decarboxylase (PDC) Activity Assay Kit

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

Operation Equipment: Spectrophotometer/Microplate reader

Cat No: AK0491

Size:100T/96S

Components:

Extract solution: 60 mL×1. Storage at 4℃ .

Reagent IA: 14 mL×1. Storage at 4℃ .

Reagent IB: Powder×1. Storage at -20℃ .

Reagent IC: 1 mL×1. Storage at 4℃ .

Reagent IIA: 3 mL×1. Storage at 4℃ .

Reagent IIB: Powder×1. Storage at -20℃ .

Reagent IIC: Powder×1. Storage at -20℃ .

Reagent III: 20 mL×1. Storage at 4℃ .

Preparation of solution：

Extract solution: Contains insoluble substance. Shake well before use.

Preparation of Reagent I: Add reagent 1B and reagent 1C to reagent 1A and dissolve thoroughly before use. Separately store at -20℃ for 1 month.

Reagent IIA: Add 0.3ml distilled water to dissolve the reagent before use, and store the inexhaustible reagent separately at -20℃ for two weeks.

Reagent IIB: Add 1ml distilled water to dissolve the reagent before use, and store the inexhaustible reagent separately at -20℃ for two weeks.

Preparation of Reagent II: 1.305ml of reagent A, 0. 12ml of reagent B and 0.075ml of reagent C were mixed (1.5mL in total, about 75T) before use.

Product Description:

Pyruvate Decarboxylase (PDC) exists in yeast mainly, which is one of the key enzymes in ethanol fermentation.

PDC catalyzes pyruvate decarboxylation to form acetaldehyde. The addition of alcohol dehydrogenase (ADH) can further catalyze the reduction of aldehydes by NADH to ethanol and NAD^+ . NADH has an absorbance at 340 nm but NAD^+ not, the activity of PDC can be calculated by measuring decrease rate of absorption at 340 nm.

Reagents and Equipment Required but Not Provided:

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

Protocol:

I. Sample extraction:

1. Bacteria:

Suggested 5 million bacteria/cell with 1 mL of Extract solution. Splitting bacteria/cell with ultrasonic (ice bath, power 20%, work time 3s, interval 10s, repeat for 30 times). Centrifuge at 16000g for 20 minutes at 4 ℃, take the supernatant and place it on ice for test.

2. Tissue:

Add 1 mL of Extract solution into 0.1 g of tissue, fully grinding on ice. Centrifuge at 16000 ×g at 4℃ for 20 minutes, take the supernatant and place it on ice for test.

3. Serum:

Detect directly.

Procedure:

1. Preheat spectrophotometer/microplate reader for 30 minutes, adjust the wavelength to 340 nm, set the counter to zero with distilled water.
2. Preheat reagent I at 37℃(mammal), 25℃(other species) in water bath for 30 minutes.
3. Add the following reagents:

Reagent name (μL)	Test tube (T)	Control tube (C)
Reagent I	100	100
Reagent III	60	60
Mixture reagent	20	20
Sample	20	-
Distilled water	-	20

Mix thoroughly, detect absorbance at 340 nm at 10s and 70s, ΔA(Test)=ΔA(T)=A1(10s)-A2(70s), ΔA(Control)=ΔA(C) =A3(10s)-A4(70s). ΔA=(A1-A2)-(A3-A4). Blank tube only needs to do 1-2 times

III. Calculation:

I. micro quartz cuvette

1. Serum:

Unit definition: One unit of enzyme activity is defined as the amount of enzyme catalyzes the oxidation of 1μmol NADH per minute at 37℃(mammal) or 25℃(other species) every milliliter of serum.

PDC(U/mL)= ΔA×Vrv÷(ε×d)×10^6÷Vs÷T=1.6×ΔA

2. Tissue:

1) Protein concentration:

Unit definition: One unit of enzyme activity is defined as the amount of enzyme catalyzes the oxidation of 1μmol NADH per minute at 37℃(mammal) or 25℃(other species) every milligram of tissue protein.

PDC (U/mg prot)= ΔA×Vrv÷(ε×d)×10^6÷(Vs×Cpr)÷T = 1.6×ΔA÷Cpr

2) Sample weight:

Unit definition: One unit of enzyme activity is defined as the amount of enzyme catalyzes the oxidation of 1μmol NADH per minute at 37℃(mammal) or 25℃(other species) every gram of tissue.

PDC (U/g)= ΔA×Vrv÷(ε×d)×10^6÷(W÷Ve ×Vs)÷T=1.6×ΔA÷W

3. Cells or bacteria:

Unit definition: One unit of enzyme activity is defined as the amount of enzyme catalyzes the oxidation of 1μmol NADH per minute at 37℃(mammal) or 25℃(other species) every 10 thousand bacteria or cells.

PDC (U/10^4 cell) =ΔA×Vrv÷(ε×d)×10^6÷(Vs÷Ve×500)÷T=3.2×10^{-3} ×ΔA÷Cpr

ε: NADH molar extinction coefficient, 6.22×10^3 L/mol/cm;

d: Light path of cuvette, 1 cm;

Vrv: Total reaction volume,0.2 mL;

Vs: Volume of supernatant added to the reaction system, 0.02 mL;

Cpr: Sample protein concentration (mg/mL); need to detect separately, suggest use PC0020, BCA Protein Assay Kit;

T: Reaction time (min), 1 minute;

W: Sample weight(g);

Ve: Extract solution volume, 1 mL;

500: amount of cell or bacteria, 5 million.

10^6: 1mol=10^6 μmol

II. 96 well flat-bottom plate

In the above formula, d- 1cm (light diameter of cuvette) is replaced by d-0.6cm (light diameter of 96 well flat-bottom plate) for calculation.

Note:

1. During the experiment, the mixture reagent, the Reagent Ⅴ and sample are placed on ice to avoid denaturation and inactivation.
2. Keep the reaction solution in the cuvette at 37℃ or 25℃ . Take a small beaker and fill it with a certain amount of distilled water at 37℃ or 25℃ Then put the beaker in a water bath at 37℃ or 25℃ . In the course of the reaction, the cuvette and the reaction liquid are put in this beaker.
3. It is better to do the experiment with two people at the same time, one person for colorimetric and the other for timing to ensure the accuracy of the experiment results.
4. React time can be extended if the change value in one minute is low, note to modify the calculation formula.

Experimental instances：

1. Take 0. 1g of Scindapsus leaf, add 1mL of extract solution, homogenate and grind. Take the supernatant, according to the measured steps, measure and calculateΔA=（A1-A2）-（A3-A4）=（0.9557-0.9299）-（0.7363-0.7301）=0.0196, calculate the enzyme activity according to sample weight:

PDC（U/g weight）=1.6×ΔA÷W=1.6×0.0196÷0. 1=0.3136 U/g weight.

2. Take 0. 1g of mouse liver, add 1mL of extract solution, homogenate and grind. Take the supernatant, 40 times dilution, according to the measured steps, measure and calculate ΔA=（A1-A2）-（A3-A4）=（0.703-0.468）-（0.7363-0.7301）=0.2288, calculate the enzyme activity according to sample weight:

PDC（U/g weight）=1.6×ΔA÷W=1.6×0.2288÷0.1×40（Dilution Ratio）=146.432 U/g weight.

References：

[1] Chong Li,Shi Gao,Xiaotong Li,et al. Efficient metabolic evolution of engineered Yarrowia lipolytica for succinic acid production using a glucose-based medium in an in situ fibrous bioreactor under low-pH condition. Biotechnology for Biofuels. August 2018;(IF5.452)

Related products：

AK0536/AK0535	Free fatty Acids(FFA) Assay Kit
AK0384/AK0383	Lipase(LPS) Activity Assay Kit
AK0297/AK0296	Plant Lipoxygenase (LOX) Assay Kit
AK0269/AK0268	Aldehyde Dehydrogenase (ALDH)