Pyruvate Phosphate Dikinase（PPDK）Activity text Kit

Detection Equipment: Spectrophotometer/Microplate Reader

Catalog Number: BC5855

Size: 100T/96S

Components:Please carefully check the volume of the reagent and the volume in the bottle before use. If you have any questions, please contact Solarbio staff in time.

Reagent name	Size	Preservation condition
Extract I	Liquid 110 mL×1	2-8℃ storage
Reagent I	Liquid 15 mL×1	2-8℃ storage
Reagent II	Powder ×1	-20℃ storage
Reagent III	Liquid 1.1 mL×1	2-8℃ storage
Reagent IV	Powder ×1	2-8℃ storage
Reagent V	Powder ×1	-20℃ storage
Reagent VI	Liquid 45 μL×1	2-8℃ storage

Solution reparation :

1. Reagent II: Add 1.1mL distilled water to fully dissolve before use. The unused reagent is storage at -20℃ for 4 weeks to avoid repeated freezing and thawing.
2. Reagent IV: Add 1.1mL distilled water to fully dissolve before use. The unused reagent is storage at -20℃ for 4 weeks to avoid repeated freezing and thawing.
3. Reagent V: Add 1.25mL distilled water to fully dissolve before use. The unused reagent is storage at -20℃ for 4 weeks to avoid repeated freezing and thawing.
4. Reagent VI working solution: Clinical use according to the sample volume according to the proportion of Reagent VI: distilled water =5μL: 120μL (a total of 125mμL, 25T), prepared for current use.
5. Preparation of Working solution: According to the sample volume before use, Reagent II: Reagent III: Reagent IV: Reagent V: Reagent VI Working solution =0.25mL: 0.25mL: 0.25mL: 0.25mL: 0.25mL(a total of 1.25mL, about 25T) is prepared into working solution for use now.

Description:

Pyruvate phosphate dikinase (PPDK, EC 2.7.9.1) is a rate-limiting enzyme in the C4 pathway and sedum acid metabolic pathway, catalyzing the three-step reaction of ATP, pyruvate and Pi to produce phosphoenolpyruvate. This enzyme mainly exists in the chloroplast matrix of C4 plants and plays an important role in regulating photosynthetic function.

The reverse reaction of PPDK catalyzes phosphoenolpyruvate (PEP), AMP and PPi to produce pyruvate, ATP and Pi, and lactate dehydrogenase (LDH) further catalyzes pyruvate and NADH to produce lactic acid and NAD+. The NADH reduction rate was measured at 340nm, according to which the PPDK activity could be calculated.

Phosphoenolpyruvate+AMP +Pyrophosphoric Acid $\xrightarrow{\text{PPDK}}$ ATP +Pi+ Pyruvic Acid

Pyruvic Acid +NADH（340nm） $\xrightarrow{\text{LDH}}$ Lactic Acid +NAD^+

Note: Before the experiment, it is recommended to select 2-3 sample with large expected differences for pre-experiment. If the absorbance value of the sample is not within the measurement range, it is recommended to dilute or increase the sample size for detection.

Reagents and Equipment Required but Not Provided:

Spectrophotometer/Microplate Reader, microquartz plate / 96-well UV plate, balance, cryogenic centrifuge, water bath, mortar/homogenizer, ice and distilled water.

Protocol:

I. Sample processing (The sample size to be tested can be appropriately adjusted, and the specific proportion can be referred to the literature)

Organization: According to tissue quality (g) : extract liquid volume (mL) is 1:5 ~10 ratio (it is

1. recommended to weigh about 0.1g tissue and add 1mL extract). Perform ice bath homogenization. 12000g, centrifuge at 4℃ for 10min, take the supernatant and put it on the ice to be measured.

II. Measurement Steps

1. It can be seen that the spectrophotometer is preheated for more than 30min, the wavelength is adjusted to340nm, and zero it with distilled water.
2. Preheat Reagent 1 at 37℃ for 10min.
3. Sample determination (These were added sequentially in microquartz cuvette / 96-well UV plate.)

Reagent name（μL）	Blank tube	Test tube
Reagent 1	650	650
Working solution	250	250
Sample	-	100
Extract	100	-

Each of the above reagents was added to a microquartz cuvette / 96-well UV plate, and the initial absorbance $A1_{blank}$ and $A1_{text}$ at 10s at 340nm were recorded after full mixing. Then the colorimetric dish and the reaction liquid were promptly placed in a 37℃ water bath for accurate reaction for 5min, and quickly removed. The absorbance $A_{blank\ 2}$ and $A_{text\ 2}$ were recorded at 340nm for 5min10s, and the $\Delta A_{text}=A_{text1}-A\ t_{ext\ 2}$, $\Delta A_{blank}=A_{blank\ 1}-A_{blank\ 2}$, $\Delta A=\Delta A_{text}-\Delta A_{blank}$. Blank tubes only need to be done 1-2 times.

III. Calculations

1. Use 96-well UV plate to determine:

1) Calculated by sample protein concentration:

Enzyme activity definition: The reduction of 1nmol NADH per mg of histone per minute in the

reaction system is defined as one unit of enzyme activity.

PPDK activity (U/mg prot) =ΔA÷ (ε×d) ×$V_{reaction}$×10^9÷(Cpr÷V_{sample})÷T×F =535.91×ΔA÷Cpr×F

2) Calculated by sample quality:

Enzyme activity definition: The reduction of 1nmol NADH per minute per g of tissue in the reaction system is defined as one unit of enzyme activity.

PPDK activity (U/g) =ΔA÷(ε×d)×$V_{reaction}$×10^9÷(W÷$V_{extraction}$×V_{sample})÷T×F = 535.91×ΔA÷W×F

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

d: Light diameter of 96-well plate, 0.6cm;

V reaction: reaction system volume, $2×10^{-4}$L;

V sample: The sample volume added to the reaction system, 0.02mL;

V extraction: Add the volume of extraction liquid, 1mL;

T: Reaction time, 5min;

Cpr: Sample protein concentration, mg/mL;

W: Sample quality, g;

F: dilution ratio;

10^9: Unit conversion, 1mol=10^9nmol.

2. Use microcuvette to determine:

The d=0.6cm in the above formula was changed to d=1cm (the optical diameter of the micro cuvette) for calculation.

Note:

1. During the measurement process, the sample and working liquid are placed on ice to avoid denaturation
and inactivation.
2. It is best for two people to do this experiment at the same time, one person to compare colors and one person to time, in order to ensure the accuracy of the experimental results.
3. When the sample ΔA < 0.005, the enzymatic reaction time can be appropriately extended or the sample size can be increased for determination; When the sample ΔA> 0.6, the supernatant can be diluted with distilled water for determination, and the dilution multiple in the calculation formula should be modified simultaneously.

Experimental example:

1. Take 0.1014g sorghum leaves and add 1mL extract solution for ice bath homogenization, take supernant and dilute it 4 times with distilled water, and then measure it with 96-well UV plate according to measure $ΔA_{blank}$ = A_{blank1}-A_{blank2}=0.754-0.749=0.005, $ΔA_{text}$ =A_{text1} - A_{text2}=1.849-1.530=0.319. ΔA=$ΔA_{text}$ - $ΔA_{blank}$ =0.319-0.005=0.314, PPDK activity calculated by sample mass:
PPDK activity (U/g mass) = 535.91×ΔA÷W×F =1549.50U/g mass.
2. Take 0.1086g grapefruit leaves and add 1mL extract solution for ice bath homogenization. Take

supernatant and follow the determination procedure.Measured with a 96-well UV plate,$\Delta A_{blank} = A_{blank1} - A_{blank2}$=0.754-0.749=0.005. $\Delta A_{text} = A_{text1} - A_{text2}$=1.849-1.530=0.319, $\Delta A = \Delta A_{text} - \Delta A_{blank}$ =0.319-0.005=0.314, PPDK activity calculated by sample mass:

PPDK activity (U/g mass) = 535.91×ΔA÷W×F =1549.50 U/g mass.

References：

[1] Chris J. Chastain and others, Functional evolution of C4 pyruvate, orthophosphate dikinase, Journal of Experimental Botany, Volume 62, Issue 9, May 2011, Pages 3083–3091.

[2] Chastain, C.J., Heck, J.W., Colquhoun, T.A. et al. Posttranslational regulation of pyruvate, orthophosphate dikinase in developing rice (Oryza sativa) seeds. Planta 224, 924–934 (2006).

[3] Aoyagi K, Bassham JA. Pyruvate orthophosphate dikinase in wheat leaves. Plant Physiol. 1983 Nov;73(3):853-4.

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