Proline Dehydrogenase (ProDH) Activity Assay Kit

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

Operation Equipment: Spectrophotometer

Cat No: BC4160

Size： 50T/48S

Components:

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

Extract solution II：0.6 mL×1. Storage at 4℃.

Reagent I: 40 mL×1. Storage at 4℃.

Reagent II: Powder×1. Storage at 4℃. When the solution will be used, add 5 mL of distilled water to fully dissolve for future use, and keep the unfinished reagent at 4℃.

Reagent III: Powder×1. Storage at 4℃. When the solution will be used, add 4 mL of distilled water to fully dissolve for future use, and keep the unfinished reagent at 4℃.

Reagent IV: Powder×1. Storage at -20℃. When the solution will be used, add 10 mL of distilled water to fully dissolve for future use, and the unused reagents can be packed separately and stored at -20℃ to avoid repeated freezing and thawing.

Product Description:

Proline Dehydrogenase (ProDH) is a key enzyme that catalyzes proline degradation in mitochondria. Reducing ProDH activity is very important for regulating osmotic balance, preventing osmotic stress from damaging plants, scavenging free radicals and protecting cell structure.

ProDH catalyzes the dehydrogenation of proline to pyruvic acid. The dehydrogenation is transferred to reduce 2,6-dichlorophenol indophenol (DCPIP) by phenazine dimethyl sulfate (PMS), and it has a characteristic absorption peak at 600 nm. The reduction rate of 2,6-DCPIP is determined by the decrease of 600 nm absorbance, which represents ProDH activity.

Reagents and Equipment Required but Not Provided:

Scales, low temperature centrifuge, spectrophotometer, 1 mL glass cuvette, acetone, homogenizer/mortar.

Procedure:

I. Sample preparation:

1. Tissue:

The mass of tissue (g): the volume of Extract solution I (mL) = 1:5~10 (it is recommended to weigh about 0.1 g tissue, add 1 mL of Extract solution I), homogenate in ice bath. Centrifuge at 1500 ×g for 10 minutes at 4℃, take the supernatant and put it into a new EP tube, add a drop of Extract solution II (add it with a 10 μL pipette tips), mix it with vortex, place it in an ice bath for 30 minutes. Centrifuge it at 15000 ×g for 20 minutes at 4℃, take the supernatant and put it on ice for testing.

2. Cells:

The number of cells (10^4): the volume of Extract solution I (mL) is 500~1000:1 (it is recommended

to add 1 mL of Extract I to 5 million cells), homogenate in ice bath. Centrifuge at 1500 ×g for 10 minutes at 4℃, take the supernatant and put it into a new EP tube, add a drop of Extract solution II (add it with a 10 μL pipette tips), mix it with vortex, place it in an ice bath for 30 minutes. Centrifuge it at 15000 ×g for 20 minutes at 4℃, take the supernatant and put it on ice for testing.

II. Determination procedure

(1) Preheat the spectrophotometer 30 minutes, adjust the wavelength to 600 nm and set zero with distilled water.

(2) Preparation of working solution: 1). Prepare Reagent III and IV into solution (see Components). 2). Before use, mix them well according to the proportion of Reagent I (V): Reagent II (V): Reagent III(V) = 1.6 (mL): 0.2 (mL): 0.15 (mL). (Note: prepare when the solution will be used. Make as much as you need), put it in 37℃ (mammal) or 25℃ (other species) water bath for 5 minutes.

(3) Add reagent table: add the following reagents to the 1 mL glass cuvette respectively.

Reagent name (μL)	Test tube (T)	Blank tube (B)
Working solution	800	800
Reagent IV	100	100
Sample	100	-
Distilled water	-	100

Add the above reagents into the 1 mL glass cuvette respectively, mix them well, measure the absorbance value at 600 nm for 10 s, quickly put them into a 37℃ (mammal) or 25℃ (other species) water bath for 3 min. Take them out and dry them quickly, measure the absorbance value at 190 s. The absorbance value of blank tube for 10s and 190s is recorded as A_1 and A_2 respectively, and that of measuring tube for 10s and 190s is recorded as A_3 and A_4 respectively. Calculate the $\Delta A_T = A_3-A_4$, $\Delta A_B = A_1-A_2$, $\Delta A = \Delta A_T-\Delta A_B$. Blank tube only needs to be done once or twice.

III. Calculation formula of enzyme activity:

(1) Calculation based on concentration of sample protein:

Unit definition: One unit of enzyme activity is defined as the amount of enzyme protein that changes the absorption value by 0.01 at 600 nm per milligram of tissue per minute in each milliliter of reaction system.

ProDH (U/mg prot) $=\Delta A \div 0.01 \times V_{RV} \div (V_S \times Cpr) \div T = 333.33 \times \Delta A \div Cpr$.

(2) Calculation based on fresh weight of sample:

Unit definition: One unit of enzyme activity is defined as the amount of enzyme of tissue that changes the absorption value by 0.01 at 600 nm per minute in each milliliter of reaction system per gram.

ProDH (U/g fresh weight) $=\Delta A \div 0.01 \times V_{RV} \div (W \times V_S \div V_{STV}) \div T = 333.33 \times \Delta A \div W$

(3) Calculation based on cell density:

Unit definition: Unit definition: One unit of enzyme activity is defined as the amount of enzyme that changes the absorption value by 0.01 at 600 nm per minute in each milliliter of reaction system per 10 thousand cells.

ProDH (U/10^4 cell) =ΔA÷0.01×V_{RV}÷(500×V_S÷V_{STV})÷T =0.67×ΔA

V_{RV}: Total volume of reaction system, 1 mL;
V_{SV}: Added the volume of sample, 0.1 mL;
V_{STV}: Added the volume of extract, 1 mL;
T: Reaction time, 3 minutes;
Cpr: Sample protein concentration, mg/mL;
W: Sample weight, g;
500: The numbers of cells or bacteria, 5 million cells.

Note；

1. When ΔA is greater than 0.3 or the value of A_3 is greater than 1.5, it is recommended to dilute the supernatant of sample with distilled water before determination.
2. Control A_3 above 0.8. If A_3 is less than 0.8, it is recommended to dilute the sample supernatant with distilled water before determination.
3. The blank tube is a test well for testing the quality of each reagent component. Under normal conditions, the change is not more than 0.02.

Experimental Examples:

1. Take 0.1g of Echinochloa crusgalli and add 1mL extract to homogenize and grind, process the sample, take the supernatant and dilute it by 2 times, and then follow the measurement procedure to calculate the ΔA t=A3-A4=0.815-0.52=0.295,ΔAb=A1-A2=0.883-0.879=0.004,ΔA=ΔAt-ΔAb=0.295-0.004=0.291, calculate the enzyme activity according to the sample quality:
ProDH (U/g mass)=333.33×ΔA÷W×dilution factor=333.33×0.291÷0.1×2=1939.981 U/g mass.
2. Take 0.1g of rabbit kidney tissue and add 1mL of extract to homogenize and grind, and process the sample. After taking the supernatant, follow the measurement procedure to obtain the calculation ΔA t=A3-A4=0.94-0.619=0.321, ΔAb =A1-A2=0.883-0.879=0.004, ΔA=ΔAt-ΔAb=0.321-0.004=0.317, the enzyme activity is calculated according to the sample quality:
ProDH (U/g mass)=333.33×ΔA÷W =333.33×0.317÷0.1=1056.656 U/g mass.

Related Products:

BC0290/BC0295 Proline(PRO) Content Assay Kit
BC1550/BC1555 Glutamic-pyruvic Transaminase(GPT) Activity Assay Kit
BC1560/BC1565 Glutamic-oxalacetic Transaminase(GOT) Activity Assay Kit