

## **Proline Dehydrogenase (ProDH) Activity Assay Kit**

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

**Operation Equipment:** Spectrophotometer/microplate reader

**Cat No:** AK0109

**Size :** 100T/96S

### **Components:**

Extract solution I : 100 mL×1. Storage at 4°C .

Extract solution II : 1.2 mL×1. Storage at 4°C .

Reagent I: 20 mL×1. Storage at 4°C .

Reagent II: Powder×1. Storage at 4°C . When the solution will be used, add 2.5 mL of distilled water to fully dissolve for future use, and keep the unfinished reagent at 4°C .

Reagent III: Powder×1. Storage at 4°C . 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°C .

Reagent IV: Powder×1. Storage at -20°C . When the solution will be used, add 5 mL of distilled water to fully dissolve for future use, and the unused reagents can be packed separately and stored at -20°C 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/microplate reader, micro glass cuvette/96 well flat-bottom plate, 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°C, 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°C, 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  $1500 \times g$  for 10 minutes at  $4^\circ\text{C}$ , take the supernatant and put it into a new EP tube, add a drop of Extract solution II (add it with a  $10 \mu\text{L}$  pipette tips), mix it with vortex, place it in an ice bath for 30 minutes. Centrifuge it at  $15000 \times g$  for 20 minutes at  $4^\circ\text{C}$ , take the supernatant and put it on ice for testing.

## II. Determination procedure

(1) Preheat the spectrophotometer/microplate reader 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  $37^\circ\text{C}$  (mammal) or  $25^\circ\text{C}$  (other species) water bath for 5 minutes.

(3) Add reagent table: add the following reagents to the micro glass cuvette/96 well plate respectively.

Reagent name ( $\mu\text{L}$ )	Test tube (T)	Blank tube (B)
Working solution	160	160
Reagent IV	20	20
Sample	20	-
Distilled water	-	20

Add the above reagents into the micro glass cuvette/96 well plate respectively, mix them well, measure the absorbance value at 600 nm for 10 s, quickly put them into a  $37^\circ\text{C}$  (mammal) or  $25^\circ\text{C}$  (other species) water bath for 3 minutes. Take them out and dry them quickly, measure the absorbance value at 190 s. (if there is a microplate reader with temperature control function, the temperature can be adjusted to  $37^\circ\text{C}/25^\circ\text{C}$ ). The absorbance value of blank tube for 10 s and 190 s 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.

## III. Calculation formula of enzyme activity:

1. Calculated according to the micro glass cuvette:

(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.

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

(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.

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

(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.

$$\text{ProDH (U/10}^4 \text{ cell)} = \Delta A \div 0.01 \times V_{RV} \div (500 \times V_S \div V_{STV}) \div T = 0.67 \times \Delta A$$

$V_{RV}$ : Total volume of reaction system, 0.2 mL;

$V_{SV}$ : Added the volume of sample, 0.02 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.

2. Calculated according to 96 well plate:

(1) Calculation based on concentration of sample protein:

Unit definition: One unit of enzyme activity is defined as the amount of per milligram of tissue protein that changes the absorption value by 0.005 at 600 nm per minute in each milliliter of reaction system.

$$\text{ProDH (U/mg prot)} = \Delta A \div 0.005 \times V_{RV} \div (V_S \times \text{Cpr}) \div T = 666.67 \times \Delta A \div \text{Cpr}$$

(2) Calculation based on fresh weight of sample:

Unit definition: One unit of enzyme activity is defined as the amount of per gram of tissue that changes the absorption value by 0.005 at 600 nm per minute in each milliliter of reaction system.

$$\text{ProDH (U/g fresh weight)} = \Delta A \div 0.005 \times V_{RV} \div (W \times V_S \div V_{STV}) \div T = 666.67 \times \Delta A \div W$$

(3) Calculation based on cell density:

Unit definition: One unit of enzyme activity is defined as the amount of per 10 thousand cells that changes the absorption value by 0.005 at 600 nm per minute in each milliliter of reaction system.

$$\text{ProDH (U/10}^4 \text{ cell)} = \Delta A \div 0.005 \times V_{RV} \div (500 \times V_S \div V_{STV}) \div T = 1.33 \times \Delta A$$

$V_{RV}$ : Total volume of reaction system, 0.2 mL;

$V_{SV}$ : Added the volume of sample, 0.02 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  $\Delta A$  is greater than 0.6 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 (96 well plate above 0.4). If  $A_3$  is less than 0.8 (when 96 well plate value is less than 0.4), 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  $\Delta A_t = A_3 - A_4 = 0.8549 - 0.4798 = 0.3751$ ,  $\Delta A_b = A_1 - A_2 = 0.8941 - 0.8813 = 0.0128$ ,  $\Delta A = \Delta A_t - \Delta A_b = 0.3751 - 0.0128 = 0.3623$ , calculate the enzyme activity according to the sample quality:

ProDH (U/g mass) =  $333.33 \times \Delta A \div W \times \text{dilution factor} = 333.33 \times 0.3623 \div 0.1 \times 2 = 2415.309$  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  $\Delta A_t = A_3 - A_4 = 0.9369 - 0.6631 = 0.2738$ ,  $\Delta A_b = A_1 - A_2 = 0.8941 - 0.8813 = 0.0128$ ,  $\Delta A = \Delta A_t - \Delta A_b = 0.2738 - 0.0128 = 0.261$ , the enzyme activity is calculated according to the sample quality:

ProDH (U/g mass) =  $333.33 \times \Delta A \div W = 333.33 \times 0.261 \div 0.1 = 869.99$  U/g mass.

**Related Products:**

AK0564/AK0563 Proline(PRO) Content Assay Kit

AK0423/AK0422 Glutamic-pyruvic Transaminase(GPT) Activity Assay Kit

AK0421/AK0420 Glutamic-oxalacetic Transaminase(GOT) Activity Assay Kit