Plant Dehydrogenase (PDHA) Activity Assay Kit

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

Operation Equipment: Microplate Reader/ Spectrophotometer

Cat No: BC3125

Size: 100T/48S

Components:

Reagent I: Powder×2. Storage at 4℃. Dissolve one bottle of powder in water to make 50 mL before use, prepare the solution when it will be use. Store at 4℃ and protect from light after prepared.

Reagent II: Liquid 100 mL×2. Storage at 4℃.

Reagent III: Ethyl acetate, required but not provided.

Product Description:

The activity of plant dehydrogenase (PDHA) is largely reflects the active state of the organism, which can directly indicate the ability of biological cells to degrade its matrix.

The hydrogen acceptor 2,3,5-triphenyl tetrazolium chloride (TTC) generates red triphenylformazone (TFF) after receiving hydrogen during cell respiration. TFF has a characteristic absorption peak at 485 nm, the PDHA activity can quantified by measuring the absorbance at 485 nm.

Reagents and Equipment Required but Not Provided:

Microplate Reader or spectrophotometer, water bath, desk centrifuge, water bath, pipette, micro glass cuvette/96 well flat-bottom plate (non-polystyrene/polypropylene material), mortar/homogenizer, ethyl acetate (express delivery is not allowed), ice and distilled water.

Procedure:

I. Complex extraction:

Collect 0.1 g of tissue, wash 3 or 4 times with double steam water, blot dry with filter paper and set aside.

II. Determination procedure:

1) Preheat spectrophotometer/ microplate reader for 30 minutes, adjust the wavelength to 485 nm, set zero with ethyl acetate.

2) Add the following reagents in 5 mL EP tubes:

Reagent	Contrast tube (Ac)	Test tube (A_T)
Sample (g)	0.1	0.1
Reagent I (mL)	-	1
Reagent II (mL)	2	1
Mix thoroughly and stand in dark for 3 hours at 37℃, ice bath for 5 minutes immediately after take out. Discard the filtrate, blot dry the sample with filter paper, place in mortar / homogenizer.		
Reagent III (mL)	1	1
Grind thoroughly (recommended to operate in a fume hood) and move all to the centrifuge tubes.		

Rinse the mortar with a small amount of Reagent III, and add the rinse solution to the centrifuge tube, supplement volume to 2 mL with Reagent III. Centrifuge at 10000 rpm for 10 minutes at 4℃, take 200 μL of supernatant in a micro glass cuvette/96 well plate to test the absorbance at 485 nm and noted as A_T, A_C, calculate $\Delta A_T=A_T-Ac$.

III. Calculation:

A. micro glass cuvette (light path of the cuvette, 1 cm)

Unit definition: One unit of enzyme activity is defined as the amount of enzyme increases the absorbance of every 0.01 for per hour every mg tissue protein in the reaction system.

PDHA Activity(U/g/h) =ΔA÷0.01÷T÷W=33×ΔA÷W

B. 96 well plate (light path of the cuvette, 0.6 cm)

Unit definition: One unit of enzyme activity is defined as the amount of 1 mg of tissue increases the absorbance of every 0.005 for per hour in the reaction system.

PDHA Activity(U/g/h) =ΔA÷0.005÷T÷W=66.7×ΔA÷W

T: Reaction time (h), 3 hours;

W: Sample weight, g.

Note

1. After prepared, Reagent I should store at 4℃, protect from light and used within one week. If it turns red, it cannot be used.
2. Reagent III is volatile and toxic. For your health, please wear lab clothes, masks and latex gloves.
3. Ice bath to stop the reaction immediately after the dark reaction was completed. Discard the filtrate, blot dry the sample with filter paper as much as possible.
4. Take two or three different samples for prediction before test. Dilute the supernatant if the absorbance is higher, multiply dilute times in the formula.
5. If test with a 96 well flat-bottom plate, polystyrene or polypropylene material 96 well flat-bottom plate is not recommended.

Experimental example:

1. Weigh 1g aloe leaves, wash them with double distilled water for 3-4 times, absorb the water with filter paper, operate according to the determination steps, measure and calculate with 96 well plate, $\Delta A=A_T-A_C$= 0.244-0.146 = 0.098, calculate the enzyme activity.

Dehydrogenase activity (U/g mass) = 66.7×ΔA÷W = 6.5366 U/g mass.

Related Products:

BC2030/BC2035	Isocitrate Lyase (ICL) Activity Assay Kit
BC3170/BC3175	Acetokinase (ACK) Activity Assay Kit
BC2010/BC2015	Glycollic Oxidase Activity Assay Kit