Polyamine Oxidase (PAO) Activity Assay Kit

Note: It is necessary to predict 2-3 large difference samples before the formal determination.

Operation Equipment: Spectrophotometer/Microplate reader

Catalog Number: BC5225

Size:100T/96S

Components:

Extract solution: Liquid 110 mL×1. Store at 2-8℃.

Reagent I: Liquid 15 mL×1. Store at 2-8℃.

Reagent II: Liquid 1.3 mL×1. Store at 2-8℃.

Reagent III: Liquid 1.3 mL×1. Store at 2-8℃.

Reagent IV: Liquid 1.1 mL×1. Store at -20℃. It could be divided into small tubules and stored at -20℃ after thawing to avoid repeated freezing and thawing.

Working solution: Reagent I, Reagent II and Reagent III are mixed by the ratio of 600μL:60μL:60μL（about 5T）to make working solution according to sample number. Prepare when the solution will be used.

Product Description:

Polyamine Oxidase (PAO) is a key enzyme which catalyse the aerobic degradation of polyamines. Polyamines have strong binding capacity to nucleic acid, protein and cell membrane molecules and directly affect cell growth, differentiation and apoptosis. PAO could be involved in the response to stress and development of plant by regulating polyamines content.

PAO catalyzes polyamines to produce H_2O_2. Peroxidase catalyzes the production of H_2O_2 into oxygen and oxidizes o-dianisidine to form colored substance which has light absorption at 500nm. The color depth is linear with PAO activity.

Reagents and Equipment Required but Not Provided:

Spectrophotometer/Microplate reader, balance, constant temperature foster box/water-bath, centrifuge, micro glass cuvette/96-well flat-bottom plate, adjustable transferpettor, mortar/homogenizer/cell ultrasonic crusher, ice and distilled water.

Procedure:

I. Sample preparation

1. **Tissue:** according to the proportion of tissue weight (g): Extract solution volume (mL) of 1:5-10 to extract. It is suggested that 0.1 g of tissue with 1 mL of Extract solution and fully homogenized on ice bath. Centrifuge at 10000 ×g for 10 minutes at 4℃ to remove insoluble materials, and take the supernatant on ice before testing.

2. **Bacteria or cells:** collect bacteria or cells into the centrifuge tube, after centrifugation discard supernatant. According to the proportion of bacteria or cells number (10^4): Extract solution

volume (mL) of 500-1000-1 to extract. It is suggested that add 1 mL of Extract solution to 5 million of bacteria or cells. Use ultrasonication to split bacteria or cells (place on ice, ultrasonic power 200W, working time 3 seconds, interval 7 seconds, repeat for 3 minutes). Centrifuge at 10000 ×g for 10 minutes at 4°C to remove insoluble materials and take the supernatant on ice for testing.

3. **Liquid:** Detect directly. Centrifuge before detect if there are precipitation in the liquid.

II. Determination

1. Preheat spectrophotometer/microplate reader for 30 min, adjust the wavelength to 500 nm and set spectrophotometer counter to zero with distilled water.
2. Preheat Working solution at 37°C for 10min.
3. Add reagents in centrifuge tube according to the following table:

Reagent（μL）	Test tube(At)	Control tube (Ac)
Working solution	140	140
Reagent IV	10	10
Mix thoroughly, stand at room temperature for 1min.		
Superatant	50	-
Distilled water	-	50
Mix thoroughly. Record the initial absorbance A1 at the wavelength of 500 nm for 30s and incubate for 1h at 37°C constant temperature foster box/water-bath. Record the absorbance A2 at the wavelength of 500 nm for 1h30s. Calculate ΔA=A2-A1. Blank tube only need to be test one or two times. **Centrifuge at 4℃ before detect if there are precipitation in the reaction solution**.		

III. Calculation:

A. 96 well flat-bottom plate:

1. Protein concentration:

Unit definition: One unit of enzyme activity is defined as the change of absorbance at the wavelength of 500 nm is 0.0005 per minute every milligram protein in the reaction system of per milliliter.

PAO activity (U/mg prot) = $\Delta A \times V_R \div V_S \div Cpr \div T \div 0.0005 \times F = 133.33 \times \Delta A \div Cpr \times F$

2. Sample weight

Unit definition: One unit of enzyme activity is defined as the change of absorbance at the wavelength of 500 nm is 0.0005 per minute every gram tissue sample in the reaction system of per gram.

PAO activity (U/g weight) = $\Delta A \times V_R \div V_S \times V_E \div W \div T \div 0.0005 \times F = 133.33 \times \Delta A \div W \times F$

3. Bacteria or cells

Unit definition: One unit of enzyme activity is defined as the change of absorbance at the wavelength of 500 nm is 0.0005 per minute every 10^4 bacteria or cells in the reaction system of per milliliter

PAO activity (U/10^4 cell) = $\Delta A \times V_R \div V_S \times V_E \div 500 \div T \div 0.0005 \times F = 0.2666 \times \Delta A \times F$

4. Liqiud

Unit definition: One unit of enzyme activity is defined as the change of absorbance at the wavelength of 500 nm is 0.0005 per minute every milliliter liqiud sample in the reaction system of per milliliter.

PAO activity (U/mL) = $\Delta A \times V_R \div V_S \div T \div 0.0005 \times F = 133.33 \times \Delta A \times F$

V_R: Total reaction volume, 0.2mL;

Vs: Add the volume of superatant, 0.05 mL;

V_E: Add the volume of Extract solution, 1 mL;

W: Sample weight, g;

Cpr: Sample protein concentration, mg/mL;

500: Cells or bacteria, 5 million;

T: Reaction time, 60 minutes;

F: Dilution times.

B. micro glass cuvette

1. Protein concentration:

Unit definition: One unit of enzyme activity is defined as the change of absorbance at the wavelength of 500 nm is 0.001 per minute every milligram protein in the reaction system of per milliliter.

PAO activity (U/mg prot) = $\Delta A \times V_R \div V_S \div Cpr \div T \div 0.001 \times F = 66.67 \times \Delta A \div Cpr \times F$

2. Sample weight

Unit definition: One unit of enzyme activity is defined as the change of absorbance at the wavelength of 500 nm is 0.001 per minute every gram tissue sample in the reaction system of per gram.

PAO activity (U/g weight) = $\Delta A \times V_R \div V_S \times V_E \div W \div T \div 0.001 \times F = 66.67 \times \Delta A \div W \times F$

3. Bacteria or cells

Unit definition: One unit of enzyme activity is defined as the change of absorbance at the wavelength of 500 nm is 0.001 per minute every 10^4 bacteria or cells in the reaction system of per milliliter

PAO activity (U/10^4 cell) = $\Delta A \times V_R \div V_S \times V_E \div 500 \div T \div 0.001 \times F = 0.133 \times \Delta A \times F$

4. Liqiud

Unit definition: One unit of enzyme activity is defined as the change of absorbance at the wavelength of 500 nm is 0.001 per minute every milliliter liqiud sample in the reaction system of per milliliter.

PAO activity (U/mL) = $\Delta A \times V_R \div V_S \div T \div 0.001 \times F = 66.67 \times \Delta A \times F$

V_R: Total reaction volume, 0.2mL;
Vs: Add the volume of superatant, 0.05 mL;
V_E: Add the volume of Extract solution, 1 mL;
W: Sample weight, g;
Cpr: Sample protein concentration, mg/mL;

500: Cells or bacteria, 5 million;
T: Reaction time, 60 minutes;
F: Dilution times.

Experimental examples:

1. Take 0.1023g *Sorbaria sorbifolia* leaf for sample processing and follow the measurement procedure. After determination with 96 well flat-bottom plate, calculate ΔAt=At2-At1=0.382-0.197=0.185. The result is calculated according to the sample mass.
PAO activity (U/g weight) = 133.33×ΔA÷W=133.33×0.185÷0.1023=241.115 U/g weight.

Related Products：

BC0090/BC0095 Peroxidase (POD) Activity Assay Kit
BC0010/BC0015 Monoamine Oxidase (MAO) Activity Assay Kit
BC1280/BC1285 Diamine Oxidase (DAO) Activity Assay Kit
BC0190/BC0195 Polyphenol Oxidase (PPO) Activity Assay Kit

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