Polyamine Oxidase (PAO) Activity Assay Kit

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

Operation Equipment: Spectrophotometer

Catalog Number: NA0167

Size:50T/48S

Components:

Extract solution: Liquid 60 mL×1. Store at 2-8℃.

Reagent I: Liquid 35 mL×1. Store at 2-8℃.

Reagent II: Liquid 3.5 mL×1. Store at 2-8℃.

Reagent III: Liquid 3.5 mL×1. Store at 2-8℃.

Reagent IV: Liquid 3 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 1T）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, balance, constant temperature foster box/water-bath, 1mL glass cuvette, adjustable transferpettor, centrifuge, 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. 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℃ 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 for 30 min, adjust the wavelength to 500 nm and set counter to zero with distilled water.
2. Preheat Working solution at 37℃ for 10min.
3. Add reagents in centrifuge tube according to the following table:

Reagent（μL）	Test tube(At)	Control tube (Ac)
Working solution	700	700
Reagent IV	50	50
Superatant	250	-
Distilled water	-	250
Mix thoroughly. Record the initial absorbance A1 at the wavelength of 500 nm for 30s and incubate for 1h at 37℃ 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:

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, 1.0mL;

Vs: Add the volume of superatant, 0.25 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 1mL glass cuvette, calculate ΔAt=At2-At1=0.637-0.256=0.381. The result is calculated according to the sample mass.

   PAO activity (U/g weight) = 66.67×ΔA÷W=66.67×0.381÷0.1023=248.302 U/g weight.

Related Products:

NA0864/NA0621	Peroxidase (POD) Activity Assay Kit
NA0871/NA0628	Monoamine Oxidase (MAO) Activity Assay Kit
NA0771/NA0530	Diamine Oxidase (DAO) Activity Assay Kit
NA0857/NA0615	Superoxide Dismutase (SOD) Activity Assay Kit