

Polyphenol Oxidase (PPO) Activity Assay Kit

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

Operation Equipment: Spectrophotometer/ Microplate reader

Cat No: NA0613

Size: 100T/48S

Components:

Extract solution: Liquid 100 mL×1, store at 4°C. Mix thoroughly with Powder I before use.

Powder I: Powder×1, store at 4°C.

Reagent I: Liquid 20 mL×1, store at 4°C.

Reagent II: Liquid 5 mL×1, store at 4°C.

Description:

Polyphenol oxidase (PPO) is mainly found in animals, plants, microorganisms and culture cells. PPO is a copper-contained oxidase that oxidizes monophenols and diphenols to produce quinones. It is closely related to fruit and vegetable processing, tea quality and tissue culture.

PPO can catalyze o-dihydroxybenzene to produce quinones which has absorbance at 410 nm.

Reagents and Equipment Required but Not Provided:

Spectrophotometer/microplate reader, refrigerated centrifuge, water bath, transferpeltor, micro glass cuvette/96 well flat-bottom plate, mortar/ homogenizer, ice and distilled water.

Protocol:

I. Sample Preparation.

1. Bacteria or cells

Collect bacteria or cells to centrifuge tube, and discard supernatant after centrifuging. Add 1 mL of Extract solution to 5 million of bacteria or cells and use ultrasonic breaking bacteria or cells. (place on ice, ultrasonic power 200W, working time 3s, interval 10s, repeat for 30 times). Centrifuge at 8000 ×g for 10 minutes at 4°C to remove insoluble materials and take the supernatant on ice for testing.

2. Tissue

Add 1 mL of Extract solution to 0.1 g of tissue, and homogenate on ice. Centrifuge at 8000 ×g for 10 minutes at 4°C to remove insoluble materials and take the supernatant on ice for testing.

II. Determination procedure.

1. Preheat spectrophotometer for 30 minutes, adjust wavelength to 410 nm, set zero with distilled water.

2. Add reagents with the following list:

Reagent (μL)	Test tube (T)	Contrast tube (C)
Reagent I	200	200

Reagent II	50	50
Sample	50	
Boiled sample		50
<p>Incubate at 37°C (mammals) or 25°C (other species) water bath for 10 minutes. Heat in boiled water for 10 minutes. Mix thoroughly, centrifuge at 5000 ×g for 10 minutes at room temperature, take the supernatant. Take 200µL of supernatant to micro glass cuvette or 96 well flat-bottom plate. Detect the absorbance of test tube and contrast tube at 410 nm, noted as A_T, A_C. $\Delta A = A_T - A_C$.</p>		

Note: Every test tube need set a contrast tube. Different samples of crude enzyme solution can be added to different contrast tubes, and then heat in boiled water for 5 minutes.

III. Calculation.

A. Micro glass cuvette

1) Protein concentration:

Unit definition: One unit of enzyme activity is defined as the amount of enzyme catalyzes the absorbance of 0.01 change at 410 nm in the reaction system per minute every milligram protein

$$\text{PPO (U/mg prot)} = \Delta A \div 0.01 \times V_{RT} \div (C_{pr} \times V_S) \div T = 60 \times \Delta A \div C_{pr}$$

2) Sample weight:

Unit definition: One unit of enzyme activity is defined as the amount of enzyme catalyzes the absorbance of 0.01 change at 410 nm in the reaction system per minute every gram tissue.

$$\text{PPO (U/g weight)} = \Delta A \div 0.01 \times V_{RT} \div (W \div V_{ST} \times V_S) \div T = 60 \times \Delta A \div W$$

3) Cells or bacteria:

Unit definition: One unit of enzyme activity is defined as the amount of enzyme catalyzes the absorbance of 0.01 change at 410 nm in the reaction system per minute every 10⁴ of cells or bacteria.

$$\text{PPO (U/10}^4 \text{ cell)} = \Delta A \div 0.01 \times V_{RT} \div (500 \div V_{ST} \times V_S) \div T = 0.12 \times \Delta A$$

B. 96 well flat-bottom plate

1) Protein concentration:

Unit definition: One unit of enzyme activity is defined as the amount of enzyme catalyzes the absorbance of 0.005 change at 410 nm in the reaction system per minute every milligram protein.

$$\text{PPO (U/mg prot)} = \Delta A \div 0.005 \times V_{RT} \div (C_{pr} \times V_S) \div T = 120 \times \Delta A \div C_{pr}$$

2) Sample weight:

Unit definition: One unit of enzyme activity is defined as the amount of enzyme catalyzes the absorbance of 0.005 change at 410 nm in the reaction system per minute every gram tissue.

$$\text{PPO (U/g)} = \Delta A \div 0.005 \times V_{RT} \div (W \div V_{ST} \times V_S) \div T = 120 \times \Delta A \div W$$

3) Cells or bacteria:

Unit definition: One unit of enzyme activity is defined as the amount of enzyme catalyzes the absorbance of 0.005 change at 410 nm in the reaction system per minute every 10⁴ of cells or bacteria.

$$\text{PPO (U/10}^4 \text{ cell)} = \Delta A \div 0.005 \times V_{RT} \div (500 \div V_{ST} \times V_S) \div T = 0.24 \times \Delta A$$

V_{RT} : Reaction total volume, 0.3 mL;
 V_S : Sample volume, 0.05 mL;
 V_{ST} : Extract solution volume, 1 mL;
 C_{pr} : Sample protein concentration, mg/mL;
 W : Sample weight, g;
500: The amount of bacteria or cells, 5 million;
 T : Reaction time, 10 minutes.

Note:

Different sample of PPO has different optimum reaction temperature, adjust temperature at 25-37°C.

Recent Product Citations:

[1] Li B, Ding Y, Tang X, et al. Effect of L-Arginine on Maintaining Storage Quality of the White Button Mushroom (*Agaricus bisporus*) [J]. Food and Bioprocess Technology, 2019, 12(4): 563-574.

[2] B Li, Y Ding, X Tang, et al. MTA1 promotes the invasion and migration of pancreatic cancer cells potentially through the HIF- α /VEGF pathway. Journal of Receptor and Signal Transduction Research. August 2018;(IF2.998)

References:

[1] González, Eva M, De Ancos B, Cano M P. Partial Characterization of Polyphenol Oxidase Activity in Raspberry Fruits[J]. Journal of Agricultural and Food Chemistry, 1999, 47(10):4068-4072.

[2] Hong - Wei Zhou, Feng X . Polyphenol oxidase from yali pear (*Pyrus bretschneideri*) [J]. Journal of the Science of Food & Agriculture, 1991, 57(3):307-313.

[3] Tang W, Newton R J. Increase of polyphenol oxidase and decrease of polyamines correlate with tissue browning in Virginia pine (*Pinus virginiana* Mill.) [J]. plant science, 2004, 167(3):621-628.

Related Products:

NA0853/NA0611 Phenylalanine Ammonialyase (PAL) Activity Assay Kit
NA0857/NA0615 Superoxide Dismutase(SOD) Activity Assay Kit
NA0854/NA0386 Catalase(CAT) Activity Assay Kit
NA0864/NA0621 Peroxidase(POD) Activity Assay Kit