

# Polyphenol Oxidase (PPO) Activity Assay Kit

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

**Operation Equipment:** Spectrophotometer

**Cat No:** BC0190

**Size:**50T/24S

## Components:

**Extract solution:** Liquid 60 mL×1, store at 4°C. Mix thoroughly with Powder I before use.

**Powder I:** Powder×1, store at 4°C.

**Reagent I:** Liquid 40 mL×1, store at 4°C.

**Reagent II:** Liquid 10 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, refrigerated centrifuge, water bath, transferpettor, 1 mL glass cuvette, 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	600	600

Reagent II	150	150
Sample	150	-
Boiled sample	-	150

Incubate at 37°C (mammals) or 25°C (other species) water bath for 10 minutes. Heat in boiled water for 10 minutes. After cooling, centrifuge at 5000 ×g for 10 minutes at room temperature, take the supernatant. Then detect the absorbance of test tube and contrast tube at 410 nm, noted as A<sub>T</sub>, A<sub>C</sub>.  
 $\Delta A = A_T - A_C$ .

**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.

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.

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

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 10<sup>4</sup> of cells or bacteria.

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

V<sub>RT</sub>: Reaction total volume, 0.9 mL;

V<sub>S</sub>: Sample volume, 0.15 mL;

V<sub>ST</sub>: Extract solution volume, 1 mL;

Cpr: 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- $\alpha$ /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:**

BC0210/BC0215 Phenylalanine Ammonialyase (PAL) Activity Assay Kit

BC0170/BC0175 Superoxide Dismutase(SOD) Activity Assay Kit

BC0200/BC0205 Catalase(CAT) Activity Assay Kit

BC0090/BC0095 Peroxidase(POD) Activity Assay Kit