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## **Polyphenol Oxidase (PPO) Activity Assay Kit**

BC1104-01(50T/24S)

**FOR RESEARCH USE ONLY, DO NOT USE IT IN CLINICAL DIAGNOSIS**

## Product 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.

## Kit components

Reagent	Volume	Storage
Extraction Solution	30mL	2-8°C, 12 months
Powder I	Powder	2-8°C, 12 months
Reagent I	40mL	2-8°C, 12 months
Reagent II	10mL	2-8°C, 12 months, in dark

**Extract solution:** Add Powder I to Extract solution before use. The solution is a suspension. Shake it before use.

## Reagents and Equipment Required but Not Provided

Spectrophotometer, refrigerated centrifuge, water bath, transferpettor, mortar/homogenizer/cell ultrasonic crusher, 1 mL glass cuvette, ice and distilled water.

## Protocol

### I. Sample Preparation

#### 1. Bacteria/Cells

Collect bacteria or cells to centrifuge tube, and discard supernatant after centrifuging. Add 1mL 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 3 seconds, interval 10 seconds, repeat for 30 times). Centrifuge at 8000 x g for 10 minutes at 4°C to remove insoluble materials and take the supernatant on ice for testing.

#### 2. Tissue:

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

#### 3. Serum (plasma) sample:

Detect sample directly. Centrifuge before detect if there are precipitation.

### II. Determination Procedures

- Preheat spectrophotometer for 30 minutes, adjust wavelength to 410 nm, set the counter to zero with distilled water.
- Add reagents with the following list:

Reagent(μL)	Test tube (T)	Blank tube (B)
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 (Wrap the sealing film to prevent bursting). After cooling, centrifuge at 5000 x 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  $A_T$ ,  $A_C$ .  $\Delta A = A_T - A_C$ .

**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.

## Calculations

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.

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

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.

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

3. Cells or bacteria number:

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  $10^4$  of cells or bacteria.

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

$V_{RT}$ : Reaction total volume, 0.9 mL;

$V_S$ : Sample volume, 0.15 mL;

$V_{ST}$ : Extract solution volume, 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.