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Ver.240802

Polyphenol Oxidase (PPO) Assay Kit

BC1104-02 (100 Tests/48 Samples)

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
Extract Solution	55 mL × 1	2-8°C
Powder I	Powder × 1	2-8°C
Reagent I	22 mL × 1	2-8°C
Reagent II	6 mL × 1	2-8°C

Solution Preparation

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

Protocol

I. Sample Preparation

- 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 3 seconds, interval 10 seconds, 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
- 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.
- Serum (plasma) sample:** detect sample directly.

II. Determination procedure.

- Preheat spectrophotometer for 30 minutes, adjust wavelength to 410 nm, set spectrophotometer counter to 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

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 \times g for 10 minutes at room temperature, take the supernatant. Take 200 μL 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 . $\Delta A = A_T - A_C$.

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

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

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.

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

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 10^4 of cells or bacteria.

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

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.

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

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.

$$\begin{aligned} \text{PPO (U/g)} &= \Delta A \div 0.005 \times V_{\text{RT}} \div (W \div V_{\text{ST}} \times V_{\text{S}}) \div T \\ &= 120 \times \Delta A \div W \end{aligned}$$

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.

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

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 samples of PPO have different optimum reaction temperature. Adjust temperature between 25 - 37°C.