(FOR RESEARCH USE ONLY. DO NOT USE IT IN CLINICAL DIAGNOSIS!)

Polyphenol Oxidase (PPO) Assay Kit

Catalog No.: BC00198

Size: 24T

Please read the instructions carefully before use. If you have any questions or need further help during experiment, please don't hesitate to contact us through the following methods:

✉ Email (Sale) order@enkilife.com

✉ Email (Techsupport) techsupport@enkilife.com

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Shelf life: Please refer to the label on the outer package.

Techsupport: In order to provide you with better service, please inform us the lot number on the label of the outer package.

Basic Information

Product Name	Polyphenol Oxidase (PPO) Assay Kit
Detection Method	Colorimetric
Sample Type	Tissue, serum, plasma, cell, bacteria
Assay Type	Quantitative
Detection Instrument	Spectrophotometer (420 nm)

Product Introduction

Polyphenol oxidase (PPO) is mainly distributed in animals, plants, microorganisms and cultured cells. As a copper-containing oxidase, it can catalyze the oxidation of monophenols and diphenols to form quinones, thereby triggering browning reactions. It is closely related to fruit and vegetable processing as well as tea quality. Meanwhile, phenolic substances can inhibit and kill pathogenic microorganisms, endowing organisms with certain disease resistance.

Principle

Polyphenol oxidase can catalyze phenolic substrates to produce quinones, which exhibit characteristic light absorption at 420 nm.

Components

No.	Components	Size	Notes
Reagent 1	Extraction solution	60 mL	Store at 4-8°C
Reagent 2	Buffer solution	40 mL	Store at 4-8°C
Reagent 3	Substrate solution	10 mL	Store at 4-8°C，protect from light.

Note: Reagent 1 must be thoroughly mixed before use; otherwise, test results will be deviated.

Storage

The reagents can be stored at 4°C for up to 3 months with stability.

Preparation

Visible spectrophotometer, 1 cm optical path cuvette (1 mL volume), centrifuge, water bath (boiling water bath included), adjustable pipette, 1 cm optical path cuvette, mortar, distilled water, ice.

- **Sample handling**

1. Tissue sample: Mix tissue fresh weight (g) with extraction solution volume (mL) at a ratio of 1:5 or 1:10 (e.g., add 1 mL of extraction solution to 0.2 g of tissue, or 1 mL of extraction solution to 0.1 g of tissue). Perform homogenization under ice-bath conditions. Centrifuge the homogenate at 8000 rpm for 10 min at room temperature; alternatively, centrifuge at 4000 rpm for 10 min, collect the supernatant, and centrifuge the supernatant again at 4000 rpm for another 10 min. Take the final supernatant for subsequent determination.
2. Serum (plasma) or fruit juice sample: Mix serum (plasma) or fruit juice volume (mL) with extraction solution volume (mL) at a ratio of 1:4 or 1:9. For example, add 0.8 mL of extraction solution to 0.2 mL of serum (plasma) or fruit juice, or add 0.9 mL of extraction solution to 0.1 mL of serum (plasma) or fruit juice. Vortex and extract for 1 minute. Centrifuge at 8000 rpm for 10 minutes at room temperature. Alternatively, centrifuge at 4000 rpm for 10 minutes, collect the supernatant, and centrifuge again at 4000 rpm for another 10 minutes. Take the supernatant for subsequent measurement.
3. Pretreatment of Bacterial or Cell Samples
   Weighing method: First weigh an empty centrifuge tube using an analytical balance (precision: 0.0001 g). Collect bacteria or cells into the centrifuge tube, centrifuge at 1000 rpm for 5 min, then discard the supernatant. Weigh the tube again and subtract the weight of the empty tube to obtain the sample mass. Add extraction solution at a ratio of 2 mg: 1000 μL or 1 mg: 1000 μL. Perform ultrasonic fragmentation under ice-bath conditions: power 20% or 200 W, ultrasonic irradiation for 3 s with a 10 s interval, repeated 30 times. Centrifuge at 8000 rpm for 10 min at 4°C, collect the supernatant for testing, and calculate the results according to the tissue calculation method.
   Cell counting method: Collect bacteria or cells into a centrifuge tube, centrifuge and discard the

supernatant. Add extraction solution at a ratio of bacterial/cell count (10^4) to extraction solution volume of 500～1000 : 1 (it is recommended to add 1 mL of extraction solution to 5 million bacteria or cells). Lyse bacteria or cells by ultrasonication under ice-bath conditions: power 20% or 200 W, ultrasonic irradiation for 3 s with a 10 s interval, repeated 30 times. Centrifuge at 8000 rpm for 10 min at room temperature, and collect the supernatant for testing.

Operation process

1. Preheat the spectrophotometer for over 30 minutes, adjust the wavelength to 420 nm, and zero the instrument with double distilled water.

	Assay tube	**Control tube**
Supernatant (μL)	150	150
Reagent 2 (μL)	600	600
Reagent 3 (μL)	150	150
Reaction conditions	Incubate accurately at a constant temperature of 37°C for 10 min, then take it out and immediately place it in a boiling water bath for 5 min.	After adding the sample and reagents, immediately place the mixture in a boiling water bath and incubate for 5 minutes.
After boiling water bath treatment, take out all tubes and cool them in ice water or room-temperature water. Centrifuge at 10000 rpm at room temperature for 10 min, then collect the supernatant. Set distilled water as blank zero adjustment, measure the absorbance value A of each tube with a spectrophotometer at 420 nm using a 1 cm optical path cuvette. Then calculate $\triangle A = A_{Assay} - A_{Control}$.		

Notes:

One assay tube and one control tube shall be set for each test sample. When using a centrifuge tube for boiling, it is recommended to pre-punch a small hole in the tube cap to prevent it from popping open.

If ($\triangle A > 0.4$), dilute the extracted supernatant by an appropriate multiple before re-measurement.

If ($\triangle A < 0.01$), increase the sample concentration or extend the reaction time of the assay tube at 37°C (e.g., 20

min or 30 min).

Calculation

1. Calculation for serum (plasma) or fruit juice samples

   Definition: One enzyme activity unit is defined as the amount of enzyme that causes a 0.01 change in absorbance at 420 nm per minute per milliliter of serum (plasma) or fruit juice in each milliliter of reaction system.

$$\text{PPO Activity (U/mL)} = \frac{\Delta A}{0.01} \times \frac{V_{Extract} + V}{V} \times \frac{V_{Total}}{V_{Sample}} \times \frac{1}{V_{Total}} \div T$$

2. Calculated based on the fresh weight of tissue samples

   Definition: One enzyme activity unit is defined as the amount of enzyme that causes a 0.01 absorbance change at 420 nm per gram of fresh tissue per minute in each milliliter of reaction system.

$$\text{PPO Activity (U/g)} = \frac{\Delta A}{0.01} \times \frac{V_{Extract}}{W} \times \frac{V_{Total}}{V_{Sample}} \times \frac{1}{V_{Total}} \div T$$

3. Calculation of tissue or bacterial (cell) samples based on protein concentration

   Definition: One enzyme activity unit is defined as a 0.01 absorbance change at 420 nm per milligram of tissue protein per minute in each milliliter of reaction system.

$$\text{PPO Activity (U/mg)} = \frac{\Delta A}{0.01} \times \frac{V_{Total}}{V_{Sample}} \times \frac{1}{V_{Total}} \div T \div Cpr$$

4. Calculation of bacterial or cell samples based on protein concentration

   Definition: One enzyme activity unit is defined as a 0.01 absorbance change at 420 nm per 10,000 bacteria or cells per minute in each milliliter of reaction system.

$$\text{PPO Activity (U/10}^4\text{)} = \frac{\Delta A}{0.01} \times \frac{V_{Extract}}{10^4} \times \frac{V_{Total}}{V_{Sample}} \times \frac{1}{V_{Total}} \div T$$

Annotation:

V: Serum (plasma) or fruit juice volume, mL

$V_{Extract}$: Total volume of extraction solution, mL

V_{Total}: Total volume of enzymatic reaction, 0.9 mL

V_{Sample}: Sample dosage, 0.15 mL

W: Sample mass, g

T: 37°C Reaction time, 10 min

Cpr: Sample protein concentration, mg/mL, shall be determined separately

10^4: Total cell count

Precautions

1. It is recommended to use disposable laboratory test tubes for operation.
2. This product is intended for scientific research use only by professionals and must not be used for clinical diagnosis or treatment, in food or drugs, or stored in ordinary residences.
3. It is critical to extract tissue/cell samples with Reagent 1 in this experiment. The crude enzyme solution after extraction shall only be used for protein determination and shall not be applied to the measurement of other indicators to avoid interference.
4. If the PPO activity of the sample is low (i.e., the absorbance difference between the assay tube and the control tube is small), extend the reaction time of the assay tube at 37°C (e.g., 20 min or 30 min), and substitute the adjusted time into the calculation formula for result computation.