



ISO 13485:2016 ISO 9001:2015

Ver.240702

Polyphenol Oxidase (PPO) Assay Kit

BC1104-01 (50 Tests/24 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.

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

Kit components

Reagent	Volume	Storage
Extract Solution	30mL × 1	2-8°C
Mix thoroughly with Powder I before use.		
Powder I	Powder × 1	2-8°C
Reagent I	40mL × 1	2-8°C
Reagent II	10mL × 1	2-8°C

Reagents and Equipment Required but Not Provided

Constant temperature water bath, cooling centrifuge, spectrophotometer, 1ml glass cuvette, mortar/homogenizer and distilled water.

Protocol

I. Sample Preparation

Tissue: Add 1mL Extract Solution to 0.1g tissue. Homogenate in ice and centrifuge at 8000 ×g at 4°C for 10 minutes. Supernatant is used for the assay. Keep the supernatant on ice.

Bacteria or cells: Add 1mL Reagent I to 5 million cells. Subject to ultrasonication while keeping the samples in an ice bath (power 200W, sonication 3s, interval 10s; repeat for 30 times). Centrifuge at 8000 ×g at 4°C for 10 minutes. Supernatant is used for the assay. Keep the supernatant on ice.

Serum/Plasma: Use directly for the assay

II. Assay procedure

- Preheat the spectrophotometer reader/ microplate reader for 30 min, adjust wavelength to 410 nm and set zero with distilled water.
- Carry out the following operation.

Reagent	Test tube (T)	Control tube(C)
Reagent I	600μL	600μL
Reagent II	150μL	150μL
Sample	150μL	-
Boiled sample	-	150μL
150μL Sample is boiled in a water bath for 10 minutes		

- Incubate at 37°C (mammals) or 25°C (other species) water bath for 10 minutes.
- Heat in a boiling water bath for 10 minutes.
- After cooling to room temperature, centrifuge at 5000 ×g for 10 minutes at room temperature, take the supernatant.
- Pipette the entire supernatant to a 1ml glass cuvette or 200μl supernatant to a micro glass cuvette or 96 well flat bottom plate and measure absorbance at 410nm
- Record absorbance A_T and A_C ; $\Delta A = A_T - A_C$

Note: Every Test needs a corresponding Control. Samples used in Control tubes has to be heated in a boiling water bath for 5 minutes.

Calculations

1. *Protein concentration:*

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

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

2. *Sample weight:*

Unit definition: One unit of enzyme is defined as the amount of enzyme which catalyzes a change of absorbance by 0.01 at 410nm in the reaction system per minute for every gram of 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*

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

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

V_{RT} : Reaction total volume, 0.9 mL

V_{S} : Sample volume, 0.15 mL

V_{ST} : 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 samples of PPO have different optimum reaction temperature. Adjust incubation temperature between 25 - 37°C according to the sample type.