CheKine™ Polyphenol Oxidase (PPO) Activity Colorimetric Assay Kit

Cat #: KTB1140 Size: 48 T/96 T

	Polyphenol Oxidase (PPO) Activity Colorimetric Assay Kit		
REF	**Cat #:** KTB1140	LOT	**Lot #:** Refer to product label
	Applicable samples: Serum, Plasma, Juices, Animal and Plant Tissues, Cells, Bacteria		
	Storage: Stored at 4℃ for 12 months, protected from light		

Assay Principle

Polyphenol Oxidase (PPO, EC1.10.3.1) is widely found in animals, plants, microorganisms and cultured cells. As a copper-containing oxidase, PPO can cause browning by oxidizing Monohydric Phenols and Dihydric Phenols to produce Quinones. This enzyme is also closely related to fruit and vegetable processing, tea quality and tissue culture. CheKine™ Polyphenol Oxidase (PPO) Activity Colorimetric Assay Kit provides a simple method for detecting PPO activity in a variety of biological samples such as Serum, Plasma, Juices, Animal and Plant Tissues, Cells and Bacteria. In the assay, Polyphenol Oxidase (PPO) catalyzes catechol to generate quinone which has a characteristic absorption peak at 525 nm.; The rate of quinone increase at 525 nm can reflect PPO activity.

Materials Supplied and Storage Conditions

Kit components	Size		Storage conditions
	48 T	96 T	
Extraction Buffer	50 mL	100 mL	4℃
Reagent Ⅰ	15 mL	30 mL	4℃
Reagent Ⅱ	5 mL	10 mL	4℃, protected from light
Reagent Ⅲ	7 mL	14 mL	4℃

Materials Required but Not Supplied

- Microplate Reader capable of measuring absorbance at OD525 nm
- Incubator
- 96 well plate with clear flat bottom, precision Pipettes, disposable Pipette Tips
- Ice Maker, Refrigerated Centrifuge
- Deionized Water
- Dounce homogenizer (for Tissue Samples)

Reagent Preparation

Extraction Buffer: Ready to use as supplied. Equilibrate to room temperature before use. Store at 4℃.

Reagent Ⅰ: Ready to use as supplied. Equilibrate to room temperature before use. Store at 4℃.

Reagent Ⅱ: Ready to use as supplied. Equilibrate to room temperature before use. Store at 4℃, protected from light.

ReagentⅢ: Ready to use as supplied. Equilibrate to room temperature before use. Store at 4℃.

Sample Preparation

1. Serum, Plasma, Juices or other Liquid Samples: According to the ratio of 0.1 mL Liquid Sample add 1 mL Extraction Buffer, homogenize on ice, Centrifuge at 8,000 g for 10 min at 4°C. Use supernatant for assay, put it on ice to be tested.

2. Animal Tissue Samples: Weigh 0.1 g Tissues, add 1 mL Assay Buffer and homogenize on ice. Centrifuge at 8,000 g for 10 min at 4℃. Use supernatant for assay, put it on ice to be tested.

3. Cells or Bacteria Samples: Collect appropriate number of Cells or Bacteria for each assay, discard the supernatant. Add 1 mL Extraction Buffer for every 5×10^6 Cells or Bacteria. Ultrasonic wave breaks Cells or Bacteria (ice bath, power 20% or 200w, ultrasonic wave 3 s, interval 10 s, repeat 30 times). Centrifuge at 8,000 g for 10 min at 4℃. Use supernatant for assay, put it on ice to be tested.

4. Plant Tissue Samples: Weigh 0.1 g Tissues, add 1 mL Assay Buffer and homogenize on ice. Centrifuge at 10,000 g for 10 min at 4℃. Use supernatant for assay, put it on ice to be tested.

Note: For additional measurement, it is recommended to use Abbkine Protein Quantification Kit (BCA Assay) (Cat #: KTD3001).

Assay Procedure

1．Preheat the Microplate Reader for more than 30 min, and adjust the wavelength to 525 nm.

2．Sample measurement (add the following reagents in sequence into the EP Tube).

3．Set the Control Tube and the Test Tube, and operate according to the Sample addition and reaction process in the following table (Operate using EP Tubes):

Reagent	Control Tube (μL)	Test Tube (μL)
Boiled Sample	50	0
Sample	0	50
Reagent Ⅰ	200	200
Reagent Ⅱ	50	50
Mix well, incubate at 25℃ (general species) or 37℃ (mammal) for 10 min, immediately place in ice bath to cool down		
Reagent Ⅲ	100	100
Mix well. Centrifuge at 5,000 g for 10 min at room temperature, use supernatant for assay.		

4．Transfer 200 μL of supernatant to desired well(s) in a 96-well plate. Measure at OD525 nm to read $A_{Control}$ and A_{Test}. Calculate $\Delta A=A_{Test}-A_{Control}$.

Note: Every Sample needs to set a Control Tube. In order to guarantee the accuracy of experimental results, need to do a pre-experiment with 2-3 samples. The optimal reaction temperature of PPO in different samples is slightly different, and it can be adjusted between 25-37℃.

Data Analysis

Note: We provide you with calculation formulae, including the derivation process and final formula. The two are exactly equal. It is suggested that the concise calculation formula in bold is final formula.

Calculation formulae based on 96-well plate are as below:

1. Calculation of PPO activity in Serum, Plasma, Fruit Juices or other Liquid Samples

Active unit definition: A 0.005 change of OD525 value per min in 1 mL Serum (Plasma) or Fruit Juice in 1 mL reaction system is defined as a unit of enzyme activity.

$PPO(U/mL)=\Delta A\times V_{Total}\div[V_{Liquid}\times V_{Sample}\div V_{Sample\ Total}]\div 0.005\div T$ **$=120\times\Delta A\div V_{Liquid}$**

2. Calculation of PPO activity in Tissues, Bacterium or Cells

(1) Calculated by protein concentration

Active unit definition: A 0.005 change of OD525 value per min in 1 mg tissue protein in 1 mL reaction system is defined as a unit of enzyme activity.

$PPO(U/mg\ prot)=\Delta A\times V_{Total}\div(V_{Sample}\times Cpr)\div 0.005\div T$ **$=120\times\Delta A\div Cpr$**

(2) Calculated by fresh weight of Samples

Active unit definition: A 0.005 change of OD525 value per min in 1 g tissue in 1 mL reaction system is defined as a unit of enzyme activity.

$PPO(U/g\ fresh\ weight)=\Delta A\times V_{Total}\div(W\times V_{Sample}\div V_{Sample\ Total})\div 0.005\div T$**$=120\times\Delta A\div W$**

(3) Calculated by Bacteria or Cell numbers

Active unit definition: A 0.005 change of OD525 value per min in 10^4 bacteria or Cells in 1 mL reaction system is defined as a unit of enzyme activity.

$PPO(U/10^4\ Cells)=\Delta A\times V_{Total}\div(500\times V_{Sample}\div V_{Sample\ Total})\div 0.005\div T$**$=0.24\times\Delta A$**

Where: V_{Total}: total reaction volume, 0.3 mL; V_{Liquid}, added volume of Serum, Plasma or Fruit Juice, 0.1 mL; V_{Sample}: sample volume added, 0.05 mL; $V_{Sample\ Total}$: volume of extract buffer added to samples, 1 mL; T: reaction time, 10 min; Cpr: sample protein concentration, mg/mL; W: sample weight, g; 500: total number of Bacteria or Cells, 5×10^6.

Recommended Products

Catalog No.	Product Name
KTB1030	CheKine™ Superoxide Dismutases (SOD) Assay Kit
KTB1040	CheKine™ Catalase (CAT) Activity Assay Kit

Disclaimer

The reagent is only used in the field of scientific research, not suitable for clinical diagnosis or other purposes.