CheKine™ Micro Plant Polyphenol Oxidase (PPO) Activity Assay Kit

Cat #: KTB1140 Size: 48 T/96 T

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

Assay Principle

Polyphenol Oxidase (PPO, EC1.10.3.1) is widely found in the plastids of plants, fungi, and insects. 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™ Micro Polyphenol Oxidase (PPO) Activity Assay Kit provides a simple method for detecting PPO activity in plant tissues. In the assay, PPO catalyzes catechol to generate quinone which has a characteristic absorption peak at 410 nm. The rate of quinone increase at 410 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

Materials Required but Not Supplied

- Microplate reader or visible spectrophotometer capable of measuring absorbance at 410 nm
- 96-well plate or microglass cuvette, precision pipettes, disposable pipette tips
- Refrigerated centrifuge, ice maker, thermostatic water bath
- Deionized water
- Dounce homogenizer

Reagent Preparation

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

Abbkine, Inc • USA • ***www.abbkine.com***
Not for further distribution without written consent.Copyright © Abbkine, Inc.

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.

Sample Preparation

Note: fresh samples are recommended. If it is not used immediately, the sample can be stored at -80℃ for one month.

1. Preparation of crude enzyme solution: treat the sample according to the ratio of plant tissue mass (g): extraction solution volume (mL) of 1: (5-10) (it is recommended to weigh 0.1 g of tissue and add 1 mL of extraction solution), homogenize 8,000 g in ice bath, centrifuge for 10 min at 4℃, take the supernatant and put it on ice for testing.
2. Boil the sample: take an appropriate amount of crude enzyme solution and boil it in a water bath for 5 min (sealed to prevent water loss), and cool it to room temperature.

Assay Procedure

1. Preheat the microplate reader or visible spectrophotometer for more than 30 min, and adjust the wavelength to 410 nm, visible spectrophotometer was returned to zero with deionized water.
2. Sample measurement (add the following reagents in sequence into the 1.5 mL EP Tube).

Reagent	Control Tube (μL)	Test Tube (μL)
Boiled Sample	50	0
Sample	0	50
Reagent Ⅰ	200	200
Reagent Ⅱ	50	50

3. Mix well. After 10 min of 25℃ water bath, quickly conduct boiling water bath for 10 min. After mixing, centrifuge at 5,000 g for 10 min at 25℃, collect the supernatant, and take 200 μL into a micro glass cuvette or 96 well plate, detect the absorbance of the measuring tube and the control tube at 410 nm, and calculate $\Delta A_{Test}=A_{Test}-A_{Control}$.

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℃. If the absorbance value of the sample is greater than 1, it is recommended to dilute it before testing.

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.

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

Active unit definition: A 0.005 change of 410 nm 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×ΔA÷W**

B. Microglass cuvette calculation formula

Active unit definition: A 0.01 change of 410 nm 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)=$\Delta A\times V_{Total}\div(W\times V_{Sample}\div V_{Sample\ Total})\div 0.01\div T$**=60×ΔA÷W**

V_{Total}: total reaction volume, 0.3 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; W: sample weight, g.

Typical Data

Abbkine, Inc • USA • *www.abbkine.com*
Not for further distribution without written consent.Copyright © Abbkine, Inc.

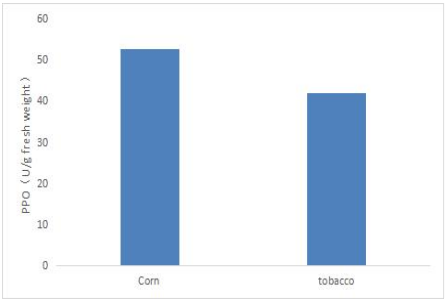

Figure 1. Determination of polyphenol oxidase activity in plant samples by this assay kit

Recommended Products

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

Not for further distribution without written consent.Copyright © Abbkine, Inc.