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CheKine™ Peroxidase (POD) Activity Assay Kit (For Plant) (Guaiacol Method)

Cat #: KTB1153 Size: 48 T/48 S 96 T/96 S

	Peroxidase (POD) Activity Assay Kit (For Plant) (Guaiacol Method)		
REF	**Cat #**: KTB1153	LOT	**Lot #:** Refer to product label
	Applicable samples: Plant Tissues		
	Storage: Stored at 4℃ for 24 months, protected from light		

Assay Principle

Peroxidase (POD, EC 1.11.1.7) is a family of redox enzymes distributed across various organelles and cellular compartments, including peroxisomes, the cytoplasm, mitochondria, the endoplasmic reticulum, lysosomes, and the plant cell wall. Using hydrogen peroxide as an electron acceptor, these enzymes catalyze the oxidation of substrates while reducing H_2O_2 to water. They also catalyze the oxidation of certain phenols, amines, aldehydes, and specific hydrocarbon oxidation products; thus, they not only facilitate the clearance of hydrogen peroxide but also participate in the metabolic transformation and detoxification of phenols, amines, aldehydes, and related compounds.

The detection principle of this assay kit is that POD catalyzes the oxidation of Guaiacol by H_2O_2 to produce a reddish-brown to tea-colored product; the activity of POD in the sample can then be quantified by measuring the change in absorbance at 470 nm.

Materials Supplied and Storage Conditions

Kit components	Size		Storage conditions
	48 T	96 T	
Reagent Ⅰ	11 mL	22 mL	4℃
Reagent Ⅱ	0.6 mL	1.2 mL	4℃, protected from light
Reagent Ⅲ	0.1 mL	0.2 mL	4℃, protected from light

Note: Before formal testing, it is recommended to select 2-3 samples with large expected differences for pre-experiment.

Materials Required but Not Supplied

- Microplate reader or visible spectrophotometer capable of measuring absorbance at 470 nm
- 96-well plate or microglass cuvette, precision pipettes, disposable pipette tips
- Incubator, ice maker, freezing centrifuge
- Deionized water, 1×PBS (pH 7.4)
- Homogenizer or mortar (for tissue samples)

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Reagent Preparation

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.

Reagent Ⅲ: 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 not assayed immediately, sub-packaged blocks can be stored at -80℃ for 1 month. Samples should be placed on ice during the assay to avoid denaturation and inactivation.

Plant Tissues: Weigh approximately 0.1 g of tissue sample, add 1 mL of 1×PBS (pH 7.4), and homogenize using a pre-cooled homogenizer equipped with an ice bath (recommended parameters: add two or three 4 mm steel beads, 55 Hz, homogenize for 30 s with 10 s intervals, repeated 6 times); then centrifuge at 8,000 g for 10 min at 4℃, collect the supernatant, and place it on ice for subsequent analysis.

Note: If the protein concentration of the sample needs to be determined, it is recommended to use Abbkine Cat #: KTD3001 Protein Quantification Kit (BCA Assay) to measure the protein concentration of the sample.

Assay Procedure

1. Preheat the microplate reader or visible spectrophotometer for more than 30 min, and adjust the wavelength to 470 nm.
2. Working Solution: Prepare immediately before use, prepare and use immediately. For each well, prepare 190 μL of Working Solution by mixing 179 μL of Reagent Ⅰ, 10 μL of Reagent Ⅱ, and 1 μL of Reagent Ⅲ, mix well.
3. Add 10 μL Sample and 190 μL Working Solution into each well of 96-well plate. Completing all sample addition steps within 5 min will not affect the test results. When testing large number of samples, it is recommended to use a multi-channel pipette to increase sample loading speed.
4. After rapid mixing, incubate for 3 min at room temperature (25℃) in the dark. Immediately read the absorbance value A_1 at 470 nm (designated as 0 min); then, after an additional 1 min of dark incubation, read the absorbance value A_2 at 470 nm (designated as 1 min). $\Delta A_{Test}=A_2-A_1$.

Note: In order to guarantee the accuracy of experimental results, it is necessary to perform a pre-experiment with 2-3 samples. If the ΔA_{Test} exceeds 0.4 or A_1 exceeds 2.0, it indicates that the sample exhibits excessively high POD activity; in such cases, the sample should be appropriately diluted with 1×PBS (pH 7.4) and re-measured. If the ΔA_{Test} is less than 0.01, the sample volume may be appropriately increased, and the assay should be repeated.

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.

1. Calculated by fresh weight of samples

Unit Definition: One unit is defined as the change of absorbance at 470 nm by 0.005 per min per g of tissue sample in per mL reaction system.

$$POD\ (U/g)=\Delta A_{Test}\times V_{Total}\div(V_{Sample}\div V_{Sample\ Total}\times W)\div 0.005\div T=\mathbf{4{,}000\times\Delta A_{Test}\div W}$$

2. Calculated by protein concentration

Unit Definition: One unit is defined as the change of absorbance at 470 nm by 0.005 per min per mg of tissue protein in per mL reaction system.

$$POD\ (U/mg\ prot)=\Delta A_{Test}\times V_{Total}\div(Cpr\times V_{Sample})\div 0.005\div T=\mathbf{4{,}000\times\Delta A_{Test}\div Cpr}$$

Where: V_{Total}: total reaction volume, 0.2 mL; V_{Sample}: sample volume added, 0.01 mL; $V_{Sample\ Total}$: 1×PBS (pH 7.4) added to samples, 1 mL; W: sample weight, g; T: reaction time, 1 min; Cpr: sample protein concentration, mg/mL.

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Typical Data

Example: Take 0.114 g of corn tissue, follow the assay procedure, and conduct the measurement using a 96-well plate. The $\Delta A_{Test}=A_2-A_1$ = 0.938 - 0.806 = 0.132. Calculating the POD activity based on sample mass: POD (U/g) = 4000 × ΔA_{Test} ÷ W = 4000 × 0.132÷0.114=4631.58 U/g

Recommended Products

Catalog No.	Product Name
KTB1010	CheKine™ Micro Coenzyme Ⅱ $NADP^+$/NADPH Assay Kit
KTB1020	CheKine™ Micro Coenzyme Ⅰ NAD^+/NADH Assay Kit
KTB1400	CheKine™ Micro Nitric Oxide (NO) Assay Kit
KTB1100	CheKine™ Micro Lactate Assay Kit
KTB1154	CheKine™ Peroxidase (POD) Activity Assay Kit (For Animal Tissue / Blood) (ABTS Method)

Disclaimer

The reagent is only used in the field of scientific research, not suitable for clinical diagnosis or other purposes. For your safety and health, please wear a lab coat and disposable gloves.

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