CheKine™ Micro Peroxidase (POD) Activity Assay Kit

Cat #: KTB1150 Size: 48 T/96 T

	Micro Peroxidase (POD) Activity Assay Kit		
REF	**Cat #**: KTB1150	LOT	**Lot #:** Refer to product label
	Applicable samples: Plants, Bacteria, Cells, Serum		
	Storage: Stored at 4℃ for 12 months, protected from light		

Assay Principle

Peroxidase, POD (EC 1.11.1.7) is widely present in animals, plants, microorganisms and cultured cells. It can catalyze hydrogen peroxide, oxidation of phenols and amine compounds, and eliminate hydrogen peroxide, phenols and amines. The dual effect of toxicity. POD catalyzes the oxidation of specific substrates by H_2O_2 to produce colored substances with characteristic light absorption at 460 nm. The kit can detect plants, bacteria, cells, serum and other samples.

Materials Supplied and Storage Conditions

Kit components	Size		Storage conditions
	48 T	96 T	
Extraction Buffer	60 mL	60 mL×2	4℃
Substrate	2.5 mL	5 mL	4℃, protected from light
H_2O_2	0.1 mL	0.2 mL	4℃, protected from light

Materials Required but Not Supplied

- Microplate reader or visible spectrophotometer capable of measuring absorbance at 460 nm
- 96-well plate or microglass cuvette, precision pipettes, disposable pipette tips
- 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℃.

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

H_2O_2: Ready to use as supplied. Equilibrate to room temperature before use. Store at 4℃, protected from light.

Sample Preparation

1. Plant: Wash cells with cold PBS and absorb water from tissues. Cut into pieces as much as you can, and then weigh them. Weigh about 0.1 g plant and add 1 mL ice-cold Extraction Buffer. For delicate plants tissues with less fiber, homogenize on ice. Centrifuge at 8,000 g for 10 min at 4℃, aspirating the supernatant, place it on ice to be tested. For plant tissues with more fibers, ultrasonically break in ice bath (power 300 W, work 3 s, intermittent 7 s, total time 3 min). Centrifuge at 8,000 g for 10 min at 4℃, aspirating the supernatant, place it on ice to be tested.
2. Bacteria or Cells samples: Collect appropriate number of bacteria or cells for each assay (initial recommendation=1-2×10^6 cells/assay). Wash the samples with cold PBS twice (Resuspend cells with PBS, centrifuge at 600 g for 10 min at 4℃). Add 1 mL Extraction Buffer for every 5×10^6 bacteria or cells. Repeated freeze-thaw cycles 2-3 times (can be frozen in liquid nitrogen, dissolved in 37℃ water bath), or process with ultrasonic disruption in ice-bath to break cells (power 300 W, ultrasound 3 s, intermittent 7 s, total time 3 min). Centrifuge at 8,000 g for 10 min at 4℃, aspirating the supernatant, place it on ice to be tested.
3. Serum or other Liquid Samples: Tested directly.

Note: If the protein concentration of the sample is need to 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 460 nm. Visible spectrophotometer was returned to zero with deionized water.
2. Working Solution: mix Extraction Buffer, Substrate, and H_2O_2 at the ratio of 139 μL: 50 μL: 1 μL per well before use (calculate according to the number of samples to be tested, and prepare more to prevent insufficient). Mix well and heat at 37℃ (mammals) or 25℃ (other species) for 10 min and to use it right after it was ready.
3. Add 10 μL Sample and 190 μL Working Solution into each well of 96-well plate or microglass cuvette. Mix well and immediately measure at OD460 nm to read the absorbance value. The absorbance value of 0 min is recorded as A_1, 1 min is A_2. $\Delta A=A_2-A_1$.

Note: In order to guarantee the accuracy of experimental results, need to do a pre-experiment with 2-3 samples. If the color becomes darker immediately after adding the working solution, the sample can be diluted with the Extraction Buffer and and repeat this assay. Multiply the results with the dilution factor. Plant samples are usually diluted 5 times.

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. 96-well Plates calculation formula

1. Calculated by fresh weight of samples

Unit Definition: One unit defines as the change of absorbance at 460 nm by 0.005 per min per g of tissue sample.

POD (U/g fresh weight) = $\Delta A\times V_{Total}\div(V_{Sample}\div V_{Sample\ Total}\times W)\div 0.005\div T=$**$4,000\times\Delta A\div W$**

2. Calculated by protein concentration

Unit Definition: One unit defines as the change of absorbance at 460 nm by 0.005 per min per mg of tissue sample.

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

3. Calculated by cells or bacteria number

Unit Definition: One unit defines as the change of absorbance at 460 nm by 0.005 per min per 10,000 bacteria or cells.

POD (U/10^4)=$\Delta A\times V_{Total}\div(500\times V_{Sample}\div V_{Sample\ Total})\div 0.005\div T=$**$8\times\Delta A$**

4. Calculated by volume of liquid samples

Unit Definition: One unit defines as the change of absorbance at 460 nm by 0.005 per min per 1 mL Liquid Sample.

POD (U/mL)=$\Delta A\times V_{Total}\div V_{Sample}\div 0.005\div T=$**$4,000\times\Delta A$**

B. Microglass cuvette calculation formula

1. Calculated by fresh weight of samples

Unit Definition: One unit defines as the change of absorbance at 460 nm by 0.01 per min per g of tissue sample.

POD (U/g fresh weight) = $\Delta A \times V_{Total} \div (V_{Sample} \div V_{Sample\ Total} \times W) \div 0.01 \div T$=**2,000×ΔA÷W**

2. Calculated by protein concentration

Unit Definition: One unit defines as the change of absorbance at 460 nm by 0.01 per min per mg of tissue sample.

POD (U/mg prot)=$\Delta A \times V_{Total} \div (Cpr \times V_{Sample}) \div 0.01 \div T$=**2,000×ΔA÷Cpr**

3. Calculated by cells or bacteria number

Unit Definition: One unit defines as the change of absorbance at 460 nm by 0.01 per min per 10,000 bacteria or cells.

POD (U/10^4)=$\Delta A \times V_{Total} \div (500 \times V_{Sample} \div V_{Sample\ Total}) \div 0.01 \div T$=**4×ΔA**

4. Calculated by volume of liquid samples

Unit Definition: One unit defines as the change of absorbance at 460 nm by 0.01 per min per 1 mL Liquid Sample.

POD (U/mL)=$\Delta A \times V_{Total} \div V_{Sample} \div 0.01 \div T$=**2,000×ΔA**

Where: V_{Total}: total reaction volume, 0.2 mL; V_{Sample}: sample volume added, 0.01 mL; $V_{Sample\ Total}$: extract buffer added to samples, 1 mL; W: sample weight, g; T: reaction time, 1 min; Cpr: sample protein concentration, mg/mL; 500: Total number of bacteria or cells, 5×10^6.

Recommended Products

Catalog No.	Product Name
KTB1030	CheKine™ Micro Superoxide Dismutases (SOD) Activity Assay Kit
KTB1040	CheKine™ Micro Catalase (CAT) Activity Assay Kit
KTB1140	CheKine™ Micro Polyphenol Oxidase (PPO) Activity Assay Kit

Disclaimer

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