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CheKine™ Peroxidase (POD) Activity Assay Kit (For Animal Tissue / Blood) (ABTS Method)

Cat #: KTB1154 Size: 48 T/48 S 96 T/96 S

	Peroxidase (POD) Activity Assay Kit (For Animal Tissue / Blood) (ABTS Method)		
REF	**Cat #**: KTB1154	LOT	**Lot #:** Refer to product label
	Applicable samples: Animal Tissues, Serum, Plasma		
	Storage: Stored at 4℃ for 12 months, protected from light		

Assay Principle

Peroxidase (POD, EC 1.11.1.7) is a class of predominantly heme-dependent redox enzymes, widely present in animal tissue cells and bodily fluids. Animals harbor diverse members of the peroxidase family, including myeloperoxidase (MPO, EC 1.11.2.2), eosinophil peroxidase (EPO), and lactoperoxidase (LPO); among these, MPO is primarily stored in the cytoplasmic granules of neutrophils and monocytes, and upon inflammatory stimulation, it can degranulate and be released into the bloodstream. These enzymes utilize H_2O_2 as the oxidizing agent to catalyze the oxidation of donor substrates such as phenols and amines, while simultaneously reducing H_2O_2 to water; thus, they participate both in H_2O_2 scavenging and in the metabolic transformation of exogenous substances. The detection principle of this assay kit is that POD catalyzes the oxidation of ABTS by H_2O_2 to generate a blue-green cationic radical, $ABTS^{\bullet+}$; the activity of POD in the sample can then be quantified by measuring the change in absorbance at 420 nm.

Materials Supplied and Storage Conditions

Kit components	Size		Storage conditions
	48 T	96 T	
Reagent Ⅰ	10 mL	20 mL	4℃
Reagent Ⅱ	3.5 mL	7 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 420 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, serum, or plasma can be stored at -80℃ for 1 month. Samples should be placed on ice during the assay to avoid denaturation and inactivation.

1.Animal 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 3 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.

2.Plasma and Serum: Tested directly. Depending on practical requirements, the sample may be diluted with 1×PBS (pH 7.4) prior to detection.

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 420 nm.

2. Working Solution: Prepare immediately before use, prepare and use immediately. For each well, prepare 196 μL of Working Solution by mixing 143 μL of Reagent Ⅰ, 52 μL of Reagent Ⅱ, and 1 μL of Reagent Ⅲ, mix well. The working solution is unstable, after preparation, it should be stored protected from light and used within 10 min.

3. Add 5 μL Sample and 195 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 420 nm (designated as 0 min); then, after an additional 1 min of dark incubation, read the absorbance value A_2 at 420 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. Samples with low enzyme activity, such as muscle and brain tissue, generally do not require dilution; however, supernatants from other animal tissues typically need to be diluted 5–10-fold with 1×PBS (pH 7.4) before analysis. It is recommended that an appropriate dilution factor be selected based on pre-experiment.

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 420 nm by 0.01 per min per g of tissue sample in per mL reaction system.

$POD\ (U/g)=\Delta A_{Test}\times V_{Total}\div(V_{Sample}\div V_{Sample\ Total}\times W)\div 0.01\div T$**$=4{,}000\times\Delta A_{Test}\div W$**

2. Calculated by volume of liquid samples

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Unit Definition: One unit is defined as the change of absorbance at 420 nm by 0.01 per min per mL Liquid sample in per mL reaction system.

POD (U/mL)=$\Delta A_{Test} \times V_{Total} \div V_{Sample} \div 0.01 \div T$**=4,000×$\Delta A_{Test}$**

3. Calculated by protein concentration

Unit Definition: One unit is defined as the change of absorbance at 420 nm by 0.01 per min per mg of tissue protein in per mL reaction system.

POD (U/mg prot)=$\Delta A_{Test} \times V_{Total} \div (Cpr \times V_{Sample}) \div 0.01 \div T$=**4,000×$\Delta A_{Test}$÷Cpr**

Where: V_{Total}: total reaction volume, 0.2 mL; V_{Sample}: sample volume added, 0.005 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.

Typical Data

Example: Take 0.105 g of rabbit liver tissue, follow the assay procedure, dilute the sample supernatant 10-fold with 1× PBS (pH 7.4), and conduct the measurement using a 96-well plate. The $\Delta A_{Test}=A_2-A_1$ = 1.025-0.915=0.110. For a rabbit liver sample diluted 10-fold, the calculation based on sample mass: POD (U/g) = 4000 × ΔA_{Test} ÷ W = 4000 × 0.110÷0.105=4190.48 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
KTB1153	CheKine™ Peroxidase (POD) Activity Assay Kit (For Plant) (Guaiacol 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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