Peroxidase (POD) Activity Assay Kit

Note: Take two or three different samples for prediction before test.

Operation Equipment: Spectrophotometer

Catalog Number: BC0090

Sizes: 50T/48S

Components:

Reagent name	Size	Preservation Condition
Extract solution	Liquid 60 mL×1	2-8℃
Reagent I	Liquid 40 mL×1	2-8℃
Reagent II	Liquid 0.04 mL×1	2-8℃
Reagent III	Liquid 10 mL×1	2-8℃

Solution Preparation:

1. Reagent II: The liquid is placed in an EP tube inside the bottle and needs to be centrifuged before use.
2. Reagent II working solution: Take 0.01 mL of reagent II and add 3.2 mL of reagent I, mix it for later use (about 24T). Prepare it for immediate use, or it can be prepared in proportion according to the sample volume.

Product Description:

Peroxidase (POD, EC 1.11.1.7) widely exists in animals, plants and microorganisms. It can catalyze the oxidation of phenols and amines by hydrogen peroxide and has the dual effect of eliminating toxicity of hydrogen peroxide, phenols and amines. In the presence of hydrogen peroxide, POD can catalyze H_2O_2 oxidize specific substrates to produce one substance which has an absorption at 470 nm.

Reagents and Equipment Required but Not Provided:

Spectrophotometer, desk centrifuge, transferpettor, mortar/homogenizer/cell ultrasonic crusher, 1 mL glass cuvette, ice and distilled water.

Procedure:

I. Sample preparation

A. Bacteria or cells

Collecting bacteria or cells into the centrifuge tube, the supernatant is discarded after centrifugation. It is suggested to take about 5 million bacteria/cell and add 1 mL of Extract solution. Bacteria or cell is splitted by ultrasonication (Power: 200 W, work time 3s, interval 10s, repeat for 30 times). Centrifuge at 8000g and 4℃ for 10 minutes, the supernatant is used for test.

B. Tissue

It is suggested to take about 0.1 g of tissue and add 1 mL of Extract solution, fully grinding on ice. Centrifuge at 8000g for 10 minutes at 4℃, the supernatant is used for test.

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C. Serum (plasma) sample: Detect sample directly.

Note: The sample homogenate supernatants are also available for BC0170/BC0175 (Superoxide Dismutase), BC5160/BC5165 (Superoxide Dismutase), BC0020/BC0025 (Malondialdehyde), BC0200/BC0205 (Catalase), BC0680/BC0685 (L-Lactate Dehydrogenase) determinations.

II. Determination procedure

1. Preheat spectrophotometer for 30 minutes, adjust wavelength to 470 nm, set zero with distilled water.
2. Place Reagent I, Reagent II working solution and Reagent III at 37℃ (mammal) or 25℃ (other species) for 10 minutes before determination.
3. Add reagents with the following list:

Reagent (μL)	Test tube
Sample	15
Distilled water	270
Reagent I	520
Reagent II working solution	130
Reagent III	135

Add the above reagents into 1 mL glass cuvette in sequence, immediately mix and time. Record the absorbance values A1 for 30 s and A2 for 90s at 470 nm and calculate ΔA＝A2-A1.

III. Calculations

I. Serum (plasma) sample

Unit definition: One unit of enzyme activity is defined as the amount of enzyme catalyzes the absorbance of 0.01 change at 470 nm in the reaction system per minute every milliliter serum(plasma).

POD activity (U/mL) =ΔA×Vrv÷Vsv÷0.01÷T =7133×ΔA

II. Tissue, bacteria or culture cells

A. Protein concentration

Unit definition: One unit of enzyme activity is defined as the amount of enzyme catalyzes the absorbance of 0.01 change at 470 nm in the reaction system per minute every milligram protein.

POD activity (U/mg prot)＝ΔA×Vrv÷(Vsv×Cpr)÷0.01÷T =7133×ΔA÷Cpr

B. Sample weight

Unit definition: One unit of enzyme activity is defined as the amount of enzyme catalyzes the absorbance of 0.01 change at 470 nm in the reaction system per minute every gram tissue.

POD activity (U/g weight)＝ΔA×Vrv÷(W× Vsv÷Vs)÷0.01÷T =7133×ΔA÷W

C. Cell amount

Unit definition: One unit of enzyme activity is defined as the amount of enzyme catalyzes the absorbance of 0.01 change at 470 nm in the reaction system per minute every 10 thousand bacteria or cells.

POD activity (U/10^4 cell)＝ΔA×Vrv÷(500×Vsv÷Vs)÷0.01÷T =14.27×ΔA

Vrv: Total reaction volume, 1.07 mL;

Vsv: Total supernatant volume, 0.015 mL;

Vs: Extract solution volume, 1 mL;

T: Reaction time, 1 minute;

Cpr: Sample protein concentration, mg/mL;

W: Sample weight, g;

500: Total number of bacteria or cells, 5 million.

Note:

1. If there are many samples to be determined at one time, the mixture of Reagent I, II working solution, III and distilled water can be prepared in proportion, and the mixture can be placed at 37℃ (mammalian) or 25℃ (other species) for more than 10 minutes. 15 μL of sample and 1055 μL of mixture can be added for determination.
2. If ΔA is less than 0.005, the reaction time can be extended to 5 minutes. If ΔA is greater than 0.5 or there are more bubbles in the reaction solution, the sample can be diluted with the extract and determined, and the calculation formula is multiplied by the corresponding dilution multiple.

Recent Product Citations:

[1] Zhang D, Liu J, Zhang Y, Wang H, Wei S, Zhang X, Zhang D, Ma H, Ding Q, Ma L. Morphophysiological, proteomic and metabolomic analyses reveal cadmium tolerance mechanism in common wheat (Triticum aestivum L.). J Hazard Mater. 2023 Mar 5; 445:130499. doi: 10.1016/j.jhazmat. 2022.130499. Epub 2022 Nov 25. PMID: 36455318.

[2] Chen Q, Cao Y, Li H, Liu H, Liu Y, Bi L, Zhao H, Jin L, Peng R. Sodium nitroprusside alleviates nanoplastics-induced developmental toxicity by suppressing apoptosis, ferroptosis and inflammation. J Environ Manage. 2023 Nov 1; 345:118702. doi: 10.1016/j.jenvman.2023.118702. Epub 2023 Aug 1. PMID: 37536135.

[3] Zhao X, Zhang Y, Ma Y, Zhang L, Jiang Y, Liang H, Wang D. Inhibitory mechanism of low-oxygen-storage treatment in postharvest internal bluing of radish (Raphanus sativus) roots. Food Chem. 2021 Dec 1;364:130423. doi: 10.1016/j.foodchem.2021.130423. Epub 2021 Jun 19. PMID: 34198034.

[4] Zhang Y, Hu Y, Wang Z, Lin X, Li Z, Ren Y, Zhao J. The translocase of the inner mitochondrial membrane 22-2 is required for mitochondrial membrane function during Arabidopsis seed development. J Exp Bot. 2023 Aug 17;74(15):4427-4448. doi: 10.1093/jxb/erad141. PMID: 37105529.

[5] Su Z, Guan K, Liu Y, Zhang H, Huang Z, Zheng M, Zhu Y, Zhang H, Song W, Li X. Developmental and behavioral toxicity assessment of opicapone in zebrafish embryos. Ecotoxicol Environ Saf. 2023 Jan 1; 249:114340. doi: 10.1016/j.ecoenv.2022.114340. Epub 2022 Dec 9. PMID: 36508804.

Referenes:

[1] Reuveni R. Peroxidase Activity as a Biochemical Marker for Resistance of Muskmelon (Cucumis melo) to Pseudoperonospora cubensis[J]. Phytopathology, 1992, 82(7).

[2] Doerge D R, Divi R L, Churchwell M I. Identification of the Colored Guaiacol Oxidation Product Produced by Peroxidases[J]. Analytical Biochemistry, 1997, 250(1):10-17.

Related Products:

BC0190/BC0195	Polyphenol Oxidase (PPO) Activity Assay Kit
BC0210/BC0215	Phenylalanine Ammonia Lyase (PAL) Activity Assay Kit
BC0170/BC0175	Superoxide Dismutase (SOD) Activity Assay Kit
BC0200/BC0205	Catalase (CAT) Activity Assay Kit

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