Peroxidase (POD) Activity Assay Kit

Note: It is necessary to predict 2-3 large difference samples before the formal determination.

Operation Equipment: Spectrophotometer/Microplate reader

Catalog Number: BC0095

Size: 100T/96S

Product Composition: Before use, please carefully check whether the volume of the reagent is consistent with the volume in the bottle. If you have any questions, please contact Solarbio staff in time.

Reagent name	Size	Preservation Condition
Extract solution	Liquid 110 mL×1	2-8℃
Reagent I	Liquid 20 mL×1	2-8℃
Reagent II	Liquid 0.04 mL×1	2-8℃
Reagent III	Liquid 3 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 106T). 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 catalyzes 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 catalyzes H_2O_2 oxidize specific substrates to produce one substance which has a absorption at 470 nm.

Reagents and Equipment Required but Not Provided：

Spectrophotometer/microplate reader, desk centrifuge, transferpettor, micro glass cuvette/96-well flat-bottom plates, mortar/homogenizer/cell ultrasonic crusher, 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 and cell is broken by ultrasonication (Power: 200W, work time 3s, interval 10s, repeat for 30 times). Centrifuge at 8000g for 10 minutes at 4℃, 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.

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/microplate reader for 30 minutes, adjust wavelength to 470 nm, set spectrophotometer to zero with distilled water.

2. Reagent I, Reagent II working solution and Reagent III is placed at 37℃ (mammal) or 25℃ (other species) for 10 minutes before determination.

3. Add reagents with the following list:

Reagent (μL)	Test tube
Reagent I	120
Reagent II working solution	30
Reagent III	30
Distilled water	60
Sample	5

The above reagents are added into EP tubes in sequence, immediately mixed and timed. Then 200 μL of the mixed solution is immediately transferred to a micro glass cuvette/ 96-well flat-bottom plates. The absorbance values A1 for 30 s and A2 for 90s at 470 nm are recorded, ΔA＝A2-A1.

III. Calculations

A. Micro glass cuvette

1. Calculate by serum (plasma) sample volume

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 actvity (U/mL) =ΔA×Vrv÷Vsv÷0.01÷T =4900×ΔA

2. Calculate by sample 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 =4900×ΔA÷Cpr

3. Calculate by 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 =4900×ΔA÷W

4. Calculate by the number of bacteria or cells

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 =9.8×ΔA

B. 96-Well flat-bottom plates

1. Calculate by serum (plasma) sample volume

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÷0.005÷T =9800×ΔA

2. Calculate by sample 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)÷0.005÷T =9800×ΔA÷Cpr

3. Calculate by 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)÷0.005÷T =9800×ΔA÷W

4. Calculate by the number of bacteria or cells

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)÷0.005÷T =19.6×ΔA

Vrv: Total reaction volume,0.245 mL;
Vsv: Total supernatant volume, 0.005 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 too many samples need test in one time, mix Reagent I, Reagent II working solution, Reagent III and distilled water in proportion. Pre-mixed solution can place at 37℃ (mammal) or 25℃ (other species) for more than 10 minutes. It is enough to add 240 μL of pre-mixed solution for text.
2. If ΔA is below 0.005, measure time can extend to 3-5 minutes. If ΔA exceed 0.8 or there are

many bubbles in the reaction solution, dilute sample with extract solution. When calculating, multiply the corresponding dilution multiple.

Recent product citations:

[1] Yin Y J, Chen C J, Guo S W, et al. The fight against Panax notoginseng root-rot disease using zingiberaceae essential oils as potential weapons[J]. Frontiers in plant science, 2018, 9: 1346.

[2] Dou S, Liu S, Xu X, et al. Octanal inhibits spore germination of Penicillium digitatum involving membrane peroxidation[J]. Protoplasma, 2017, 254(4): 1539-1545.

[3] Li B, Ding Y, Tang X, et al. Effect of L-Arginine on Maintaining Storage Quality of the White Button Mushroom (Agaricus bisporus) [J]. Food and Bioprocess Technology, 2019, 12(4): 563-574.

[4] Yanan Wang,Chengzhen Liang,Zhigang Meng,et al. Leveraging Atriplex hortensis choline monooxygenase to improve chilling tolerance in cotton. Environmental and Experimental Botany. June 2019; 162:364-373.(IF3.712)

[5] Yanjiao Yin,Chuanjiao Chen,Shiwei Guo,et al. The Fight Against Panax notoginseng Root-Rot Disease Using Zingiberaceae Essential Oils as Potential Weapons. Frontier in Immunology. October 2018;(IF4.716)

References:

[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