Peroxidase (POD) Activity Assay Kit

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

Operation Equipment: Spectrophotometer/Microplate reader

Catalog Number: BC0095

Size: 100T/96S

Components:

Extract solution:110 mL×1. Storage at 4℃.

Reagent I: 20 mL×1. Storage at 4℃.

Reagent II: 0.21 mL×1. Storage at 4℃. Centrifuge before use. Take 0.1 mL of reagent II and add 1.5mL of reagent I and mix it for later use. Prepare it for immediate use, or it can be prepared in proportion according to the sample volume.

Reagent III: 3 mL×1. Storage at 4℃.

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//homogeniser, 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: 20%, work time 3s, interval 10s, repeat for 30 times). Centrifuge at 8000 rpm 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 8000 rpm for 10 minutes at 4℃, the supernatant is used for test.

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

II. Determination procedure：

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

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

minutes before determination.

3. Add reagents with the following list:

Name of reagent (μL)	Test tube
Reagent I	120
Reagent II	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. 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(U/mL)=ΔA×Vrv÷Vsv÷0.01÷T =4900×ΔA

2. 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(U/mg prot)＝ΔA×Vrv÷(Vsv×Cpr)÷0.01÷T =4900×ΔA÷Cpr

3. 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(U/g fresh weight)＝ΔA×Vrv÷(W× Vsv÷Vs)÷0.01÷T =4900×ΔA÷W

4. 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(U/10^4 cell)＝ΔA×Vrv÷(500×Vsv÷Vs)÷0.01÷T =9.8×ΔA

B. 96-Well flat-bottom plates

1. 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(U/mL)=ΔA×Vrv÷Vsv÷0.005÷T =9800×ΔA

2. 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(U/mg prot)＝ΔA×Vrv÷(Vsv×Cpr)÷0.005÷T =9800×ΔA÷Cpr

3. 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(U/g fresh weight)＝ΔA×Vrv÷(W× Vsv÷Vs)÷0.005÷T =9800×ΔA÷W

4. 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(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 much samples need test in one time, mix Reagent I, Reagent II, 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 0.5, 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