Ver.251201

Peroxidase (POD) Activity Assay Kit

BC1103-02(100T/96S)

FOR RESEARCH USE ONLY, DO NOT USE IT IN CLINICAL DIAGNOSIS

Product Description

Peroxidase 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.

Kit components

Reagent	Volume	Storage
Extraction Reagent	110mL	2-8°C
Reagent I	20mL	2-8°C
Reagent II	25μL	2-8°C
Reagent III	3mL	2-8°C

Reagent 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. Prepare it for immediate use, or it can be prepared in proportion according to the sample volume.

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.

Operation Procedures

I. Sample Preparation

1. 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°C, the supernatant is used for test.

2. Tissue

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

3. Serum or Plasma

Directly use for the assay.

II. Determination procedure

1. Preheat spectrophotometer/microplate reader for 30 minutes, adjust wavelength to 4/0 nm, set spectrophotometer to zero with distilled water.
2. Reagent I, Reagent II working solution and Reagent III is placed at 37°C (mammal) or 25°C (other species) for 10 minutes before determination.
3. Add reagents with the following list:

Reagent	Test tube (μL)
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.

Calculation

1. Protein concentration:

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

POD (U/mg protein) = ΔA × Vrv ÷ (Vsv × Cpr) ÷ 0.01 ÷ T

= 4900×ΔA÷Cpr

2. Sample weight:

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

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

= 4900×ΔA÷W

3. Cells

Unit definition: One unit of enzyme activity is defined as the amount of enzyme that catalyzes change of absorbance by 0.01 at 470 nm in the reaction system per minute for every 10^4 cells or bacteria.

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

= 9.8×ΔA

4. Serum/Plasma:

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

POD (U/mL) = ΔA×Vrv÷Vsv÷0.01÷T

= 4900×ΔA

Vrv : Reaction total volume, 1.07 mL
Vsv : Sample volume, 0.015 mL
V_S : Extract solution volume, 1 mL
Cpr : Sample protein concentration, mg/mL
W : Sample weight, g
500 : The amount of bacteria or cells, 5 million
T : Reaction time, 1 minute.

Notes:

1. If ΔA is below 0.005, measure time can extend to 3-5 minutes, correspondingly the change has to be incorporated into the equation during calculation. If ΔA exceed 0.5, dilute sample with extract solution. When calculating, multiply the corresponding dilution factor.

2. If many samples are being assayed at once, a mixture of Reagent I, II III and distilled water can be prepared in proportion and placed at 37℃ (mammal) or 25℃ (other species) for 10 minutes before starting the experiment. 15μL sample and 1055μL mixture be used during the assay.