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Peroxidase (POD) Activity Assay Kit

BC1103-01 (50 Tests/48 Samples)

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

Product Description

Peroxidase 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 catalyzes H_2O_2 oxidize specific substrates to produce one substance which has a absorption at 470 nm.

Kit components

Reagent	Volume	Storage
Extract Solution	60mL \times 1	4°C
Reagent I	40mL \times 1	4°C
Reagent II	0.04 mL \times 1	4°C
Spin down the contents of the tube. Take 0.01 mL of Reagent II and mix with 3.2 mL Reagent I. This will be sufficient for 24 tests. Prepare immediately before use.		
Reagent III	10mL \times 1	4°C

Reagents and Equipment Required but Not Provided

Constant temperature water bath, spectrophotometer, 1ml glass cuvette, mortar/homogenizer and distilled water.

Protocol

I. Sample Preparation

Tissue: Add 1mL Extract Solution to 0.1g tissue. Homogenate in ice and centrifuge at $8000 \times g$ at 4°C for 10 minutes. Supernatant is used for the assay. Keep the supernatant on ice.

Bacteria or cells: Add 1mL Extract Solution to 5 million cells. Subject to ultrasonication while keeping the samples in an ice bath (power 200W, sonication 3 seconds, interval 10 seconds; repeat for 30 times). Centrifuge at $8000 \times g$ at 4°C for 10 minutes. Supernatant is used for the assay. Keep the supernatant on ice.

Serum/Plasma: Use directly for the assay.

II. Assay procedure

- Preheat the spectrophotometer reader/ microplate reader for 30 minutes, adjust wavelength to 470 nm and set zero with distilled water.
- Place Reagent I, Reagent II and Reagent III at 37°C (mammal) or 25°C (other species) for 10 minutes before starting the experiment.
- Carry out the following operation.

Reagent	Test tube (T)
Sample	15 μ L
Distilled Water	270 μ L
Reagent I	520 μ L
Reagent II	130 μ L
Reagent III	135 μ L

- The reagents and sample are added to 1 mL glass cuvette in sequence.
- Mix thoroughly and measure absorbance A_1 at 30 seconds and A_2 at 90 seconds at 470nm
- $\Delta A = A_2 - A_1$

Note: Every Test needs a corresponding Control. Samples used in Control tubes has to be heated in a boiling water bath for 5 minutes.

Calculations

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.

$$\text{POD (U/mg protein)} = \Delta A \times V_{rv} \div (V_{sv} \times C_{pr}) \div 0.01 \div T \\ = 7133 \times \Delta A \div C_{pr}$$

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.

$$\text{POD (U/g weight)} = \Delta A \times V_{rv} \div (W \times V_{sv} \div V_s) \div 0.01 \div T \\ = 7133 \times \Delta A \div W$$

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.

$$\text{POD (U/10}^4 \text{ cells)} = \Delta A \times V_{rv} \div (500 \times V_{sv} \div V_s) \div 0.01 \div T \\ = 14.27 \times \Delta A$$

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.

$$\text{POD (U/mL)} = \Delta A \times V_{rv} \div V_{sv} \div 0.01 \div T \\ = 7133 \times \Delta A$$

V_{rv} : Reaction total volume, 1.07 mL

V_{sv} : Sample volume, 0.015 mL

V_s : Extract solution volume, 1 mL

C_{pr} : Sample protein concentration, mg/mL

W : Sample weight, g

500 : The amount of bacteria or cells, 5 million

T : Reaction time, 1 minute.

Note:

- 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.
- 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°C (mammal) or 25°C (other species) for 10 minutes before starting the experiment. 15µL sample and 1055µL mixture be used during the assay.