Peroxidase (POD) Activity Assay Kit
(Serum or Plasma Samples)

Catalog No: E-BC-K226-S

Method: Colorimetric method

Specification: 50Assays (Can detect 48 samples without duplication)

Measuring instrument: Spectrophotometer

Sensitivity: 0.5 U/mL

Detection range: 0.5-300U/mL

- Please kindly provide us the lot number (on the outside of the box) of the kit for more efficient service.

General information

▲Intended use

This kit can be used to measure the POD activity in animal serum or plasma samples.

▲Background

Peroxidase is a kind of oxidoreductase. Distributed in breast milk, white blood cells, platelets and other body fluids or cells, the prosthetic group of the enzyme is also heme, the enzyme that uses H_2O_2 as the electron acceptor to catalyze the oxidation of the substrate, it catalyzes the direct oxidation of phenolic or amine compounds by H_2O_2, such as glutathione peroxidase, eosinophil peroxidase and thyroid peroxidase, etc., have the dual effect of eliminating the toxicity of hydrogen peroxide and phenolic amines.

▲Detection principle

This kit is based on the reaction of hydrogen peroxide catalyzed by peroxidase, the POD activity can be calculated by measuring the change in absorbance at 420nm.

▲Kit components & storage

Item	Component	Specification	Storage
Reagent 1	Liquid	60 mL × 2 vials	2-8℃ , 6 months
Reagent 2	Powder	2 vials	2-8℃ , 6 months, shading light
Reagent 3	Liquid	5 mL × 1 vial	2-8℃ , 6 months
Reagent 4	Liquid	50 mL × 1 vial	2-8℃ , 6 months
Note: The reagents must be stored strictly according to the preservation conditions in the above table. The reagents in different kits cannot be mixed with each other.			

▲Materials prepared by users

Instruments

Spectrophotometer (240nm&420nm), Micropipettor, Vortex mixer, Centrifuge, Water bath, Incubator

Consumptive material

Tips (10 μL, 200 μL, 1000 μL), EP tubes (1.5 mL, 2 mL)

Reagents

Double distilled water

▲Safety data

Some of the reagents in the kit contain dangerous substances. It should be avoided to touch the skin and clothing. Wash immediately with plenty of water if touching it carelessly. All the samples and waste material should be treated according to the relevant rules of laboratory's biosafety.

▲Precautions

Before the experiment, please read the instructions carefully, and wear gloves and work clothes.

▲The key points of the assay

1. The reaction time must be controlled strictly.
2. The light should be prevented during the experiment, so as to avoid the phenomenon that the difference between the multiple wells is too large.
3. Don't take the precipitate when take the supernatant for measuring the OD value to avoid the effect of precipitate to OD value.
4. The step of measuring the OD value must be finished in 30 min.
5. During the detection, the cuvettes should be washed, so as to avoid the residual water in the cuvette to affect the results.

Pre-assay preparation

▲Reagent preparation

1. Preparation of reagent 2 application solution:

Dissolve a vial of reagent 2 with 10 mL of double distilled water before use. The prepared solution can be stored at 4°C with shading light.

2. Preparation of reagent 3 application solution:

Dilute the reagent 3 for 15 times with double distilled water before use. Measure the OD value at 240 nm with 1 cm optical path cuvette (set the spectrophotometer to zero with double distilled water). If the OD value is about 0.4, then the reagent 3 application solution is prepared. If the OD value is too high, then dilute the reagent with double distilled water. If the OD value is too low, then add appropriate amount of reagent 3. (Generally, the dilution ratio is 25.)

▲Dilution of sample

It is recommended to take 2~3 samples with expected large difference to do pre-experiment before formal experiment and dilute the sample according to the result of the pre-experiment and the detection range (0.5-300U/mL).

Assay protocol	
Ambient temperature	25-30°C
Optimum detection wavelength	420 nm

Instructions for the use of transferpettor:

(1) Equilibrate the pipette tip in that reagent before pipetting each reagent.

(2) Don't add the liquid outside the tips into the reaction system when pipetting each reagent.

Assay protocol

▲Operating steps

1. Add 2.4 mL of reagent 1, 0.3 mL of reagent 2 application solution and 0.2 mL of reagent 3 application solution to each tube.
2. Blank tube: add 0.1 mL of double distilled water to the tube.
   Sample tube: add 0.1 mL of sample to the tube.
3. Incubate at 37 ℃ for 30 min accurately.
4. Add 1 mL of reagent 4 to each tube.
5. Mix fully, centrifuge at 3500 rpm for 10 min and take the supernatant. Set the spectrophotometer to zero with double distilled water and measure the OD values of each tube at 420 nm with 1cm optical path cuvette.

▲Operation table

	Blank tube	Sample tube
Reagent 1 (mL)	2.4	2.4
Reagent 2 application solution (mL)	0.3	0.3
Reagent 3 application solution (mL)	0.2	0.2
Double distilled water (mL)	0.1	
Sample (mL)		0.1
Incubate at 37°C for 30 min accurately.		
Reagent 4 (mL)	1.0	1.0
Mix fully, centrifuge at 3500 rpm for 10 min and take the supernatant. Set the spectrophotometer to zero with double distilled water and measure the OD values of each tube at 420 nm with 1cm optical path cuvette.		

▲Calculation

1. Definition

The enzyme amount that 1 μg substrate catalyzed by 1 mL of sample per minute at 37°C is defined as 1 unit.

2. Calculation formula

$$\text{POD activity (U/mL)} = \frac{\Delta A}{12 \times d} \times \frac{V_{total}}{V_{sample}} \times 1000 \div t \times f$$

Note:

ΔA: $OD_{Sample} - OD_{Blank}$

d: The optical path of the cuvette, 1 cm

V_{total}: the total volume of reaction, mL.

V_{sample}: the volume of sample added into the reaction system, mL.

t: reaction time, 30 min.

f: Dilution factor of sample before test.

12: Constant.

1000: Constant.

▲Notes

1. This kit is for research use only.
2. Instructions should be followed strictly, changes of operation may result in unreliable results.
3. The validity of kit is 6 months.
4. Do not use components from different batches of kit.