（FOR RESEARCH USE ONLY. DO NOT USE IT IN CLINICAL DIAGNOSIS !）

Catalog No：E-BC-K226-S

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

Measuring instrument：Spectrophotometer

Detection range：0.5-300U/mL

Elabscience®Peroxidase (POD) Activity Assay Kit

(Serum or Plasma Samples)

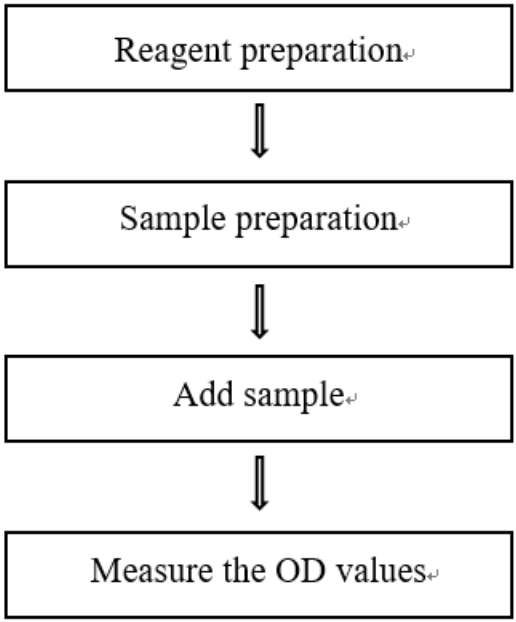
This manual must be read attentively and completely before using this product.
If you have any problem, please contact our Technical Service Center for help：

Toll-free: 1-888-852-8623
Tel: 1-832-243-6086
Fax: 1-832-243-6017
Email: techsupport@elabscience.com
Website: www.elabscience.com

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

Table of contents

Assay summary



Intended use

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

Detection principle

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.

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℃, 12 months
Reagent 2	Powder	2 vials	2-8℃, 12 months, shading light
Reagent 3	Liquid	5 mL ×1 vial	2-8℃, 12 months
Reagent 4	Liquid	50 mL ×1 vial	2-8℃, 12 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. For a small volume of reagents, please centrifuge before use, so as not to obtain sufficient amount of reagents.

Materials prepared by users

Instruments:

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

Reagents:

Double distilled water

Reagent preparation

① Equilibrate all reagents to room temperature before use.

② 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℃ with shading light.

③ 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.)

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.

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.

Calculation

The sample:

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

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

[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.

Appendix I Standard curve

Reagent and preparation

Dilute the POD standard (self-prepared) to different concentrations with double distilled water.

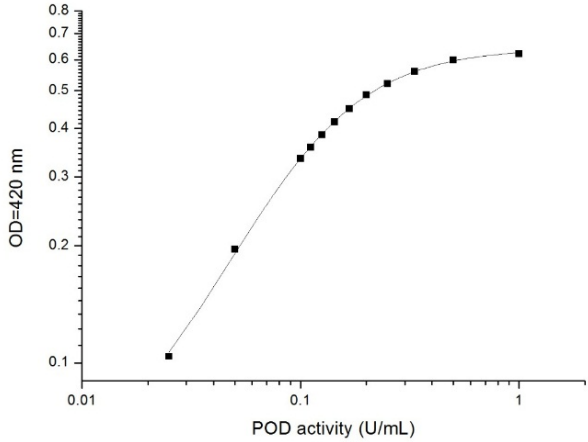
Operation table

	Blank tube	Standard 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	
POD standard solution with different concentrations (mL)		0.1
Incubate for accurately 30 min at 37℃.		
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.		

Detection results

OD_{Blank}	0.113											
Activity unit	0.025	0.05	0.1	0.111	0.125	0.143	0.167	0.2	0.25	0.333	0.5	1
OD	0.217	0.309	0.447	0.47	0.498	0.529	0.562	0.6	0.634	0.673	0.712	0.735
Absolute OD	0.104	0.196	0.334	0.357	0.385	0.416	0.449	0.487	0.521	0.56	0.599	0.622

Standard curve (for reference only)



Statement

1. This assay kit is for Research Use Only. We will not response for any arising problems or legal responsibilities causing by using the kit for clinical diagnosis or other purpose.
2. Please read the instructions carefully and adjust the instruments before the experiments. Please follow the instructions strictly during the experiments.
3. Protection methods must be taken by wearing lab coat and latex gloves.
4. If the concentration of substance is not within the detection range exactly, an extra dilution or concentration should be taken for the sample.
5. It is recommended to take a pre-test if your sample is not listed in the instruction book.
6. The experimental results are closely related to the situation of reagents, operations, environment and so on. Elabscience will guarantee the quality of the kits only, and NOT be responsible for the sample consumption caused by using the assay kits. It is better to calculate the possible usage of sample and reserve sufficient samples before use.