

Peroxidase (POD) Activity Colorimetric Assay Kit

Cat #: orb1173210 (manual)

Product Features

Product name: Peroxidase (POD) Activity Colorimetric Assay Kit

Catalog number: orb1173210

Sample Types: Animal and Plant Tissues, Bacteria, Cells, Serum

Storage: Stored at 4°C for 12 months, protected from light.

Assay Principle

Peroxidase, POD (EC 1.11.1.7) is widely present in animals, plants, microorganisms and cultured cells. It can catalyze hydrogen peroxide, oxidation of phenols and amine compounds, and eliminate hydrogen peroxide, phenols and amines. The dual effect of toxicity. POD catalyzes the oxidation of specific substrates by H₂O₂ to produce colored substances with characteristic light absorption at 460 nm. The kit can detect plants, bacteria, cells, serum and other samples.

Kit Components

Kit components	Size		Storage conditions
	48 T	96 T	
Extraction Buffer	70 mL	70 mL×2	4°C
Substrate	3 mL	6 mL	4°C, protected from light
H ₂ O ₂	0.1 mL	0.2 mL	4°C, protected from light

Note: Before formal testing, it is recommended to select 2-3 samples with large, expected differences for pre-experiment.

Materials Required but Not Supplied

- Microplate reader or visible spectrophotometer capable of measuring absorbance at 460 nm
- 96-well plate or microglass cuvette, precision pipettes, disposable pipette tips
- Deionized water
- Dounce homogenizer (for tissue samples)

Reagent Preparation

Extraction Buffer: Ready to use as supplied. Equilibrate to room temperature before use. Store at 4°C.

Substrate: Ready to use as supplied. Equilibrate to room temperature before use. Store at 4°C, protected from light.

H2O2: Ready to use as supplied. Equilibrate to room temperature before use. Store at 4°C, protected from light.

Sample Preparation

Note: Fresh samples are recommended, if not assayed immediately, samples can be stored at -80°C for one month.

1. **Animal Tissues:** Wash tissues with cold PBS and absorb water. Weigh about 0.1 g tissues and add 1 mL ice-cold Extraction Buffer to homogenize on ice. Centrifuge at 8,000 g for 10 min at 4°C, aspirating the supernatant, place it on ice to be tested.
2. **Plant Tissues:** Wash tissues with cold PBS and absorb water. Cut into pieces as much as you can and then weigh them. Weigh about 0.1 g plant and add 1 mL ice-cold Extraction Buffer. For delicate plants tissues with less fiber, homogenize on ice. Centrifuge at 8,000 g for 10 min at 4°C, aspirating the supernatant, place it on ice to be tested. For plant tissues with more fibers, ultrasonically break in ice bath (power 300 W, work 3 s, intermittent 7 s, total time 3 min). Centrifuge at 8,000 g for 10 min at 4°C, aspirating the supernatant, place it on ice to be tested.
3. **Bacteria or Cells samples:** Collect appropriate number of bacteria or cells for each assay (initial recommendation=1-2×10⁶ cells or bacteria/assay). Wash the samples with cold PBS twice (Resuspend cells with PBS, centrifuge at 600 g for 10 min at 4°C). Add 1 mL Extraction Buffer for every 5×10⁶ bacteria or cells and process with ultrasonic disruption in ice-bath to break cells (power 300 W, ultrasound 3 s, intermittent 7 s, total time 3 min). Centrifuge at 8,000 g for 10 min at 4°C, aspirating the supernatant, place it on ice to be tested.
4. **Serum or other Liquid Samples:** Tested directly.

Note: If the protein concentration of the sample needs to be determined, it is recommended to use Cat #: orb1147876 Protein Quantification Kit (BCA Assay) to measure the protein concentration of the sample.

Assay Procedure

1. Preheat the microplate reader or visible spectrophotometer for more than 30 min and adjust the wavelength to 460 nm. Visible spectrophotometer was returned to zero with deionized water.
2. **Working Solution:** mix Extraction Buffer, Substrate, and H2O2 at the ratio of 139 µL: 50 µL: 1 µL per well before use (calculate according to the number of samples to be tested and prepare more to prevent insufficient). Mix well and heat at 37°C (mammals) or 25°C (other species) for 10 min and to use it right after it was ready.
3. Add 10 µL Sample and 190 µL Working Solution into each well of 96-well plate or microglass cuvette. Mix well and immediately measure at OD460 nm to read the absorbance value. The absorbance value of 0 min is recorded as A1, 1 min is A2. $\Delta A = A2 - A1$. Note: To guarantee the accuracy of experimental results, need to do a pre-experiment with 2-3 samples. If the color becomes darker immediately after adding the working solution or ΔA_{Test} is greater than 1.0, the sample can be diluted with the Extraction Buffer and and repeat this assay. Multiply the results with the dilution factor. If ΔA_{Test} is less than 0.005, the reaction time can be extended to 5-10 min or the sample volume can be appropriately increased. Plant samples are usually diluted 5 times.

Calculation of Results

Note: We provide you with calculation formulae, including the derivation process and final formula. The two are exactly equal. It is suggested that the concise calculation formula in bold is final formula.

A. 96-well Plates calculation formula

1. Calculated by fresh weight of samples

Unit Definition: One unit defines as the change of absorbance at 460 nm by 0.005 per min per g of tissue sample. $POD (U/g \text{ fresh weight}) = \Delta A \times V_{Total} \div (V_{Sample} \div V_{Sample \text{ Total}} \times W) \div 0.005 \div T = 4,000 \times \Delta A \div W$

2. Calculated by protein concentration

Unit Definition: One unit defines as the change of absorbance at 460 nm by 0.005 per min per mg of tissue sample. $POD (U/mg \text{ prot}) = \Delta A \times V_{Total} \div (Cpr \times V_{Sample}) \div 0.005 \div T = 4,000 \times \Delta A \div Cpr$

3. Calculated by cells or bacteria number

Unit Definition: One unit defines as the change of absorbance at 460 nm by 0.005 per min per 10,000 bacteria or cells.

$$POD (U/10^4) = \Delta A \times V_{Total} \div (500 \times V_{Sample} \div V_{Sample \text{ Total}}) \div 0.005 \div T = 8 \times \Delta A$$

4. Calculated by volume of liquid samples

Unit Definition: One unit defines as the change of absorbance at 460 nm by 0.005 per min per 1 mL Liquid Sample.

$$POD (U/mL) = \Delta A \times V_{Total} \div V_{Sample} \div 0.005 \div T = 4,000 \times \Delta A$$

B. Microglass cuvette calculation formula

1. Calculated by fresh weight of samples

Unit Definition: One unit defines as the change of absorbance at 460 nm by 0.01 per min per g of tissue sample. $POD (U/g \text{ fresh weight}) = \Delta A \times V_{Total} \div (V_{Sample} \div V_{Sample \text{ Total}} \times W) \div 0.01 \div T = 2,000 \times \Delta A \div W$

2. Calculated by protein concentration

Unit Definition: One unit defines as the change of absorbance at 460 nm by 0.01 per min per mg of tissue sample. $POD (U/mg \text{ prot}) = \Delta A \times V_{Total} \div (Cpr \times V_{Sample}) \div 0.01 \div T = 2,000 \times \Delta A \div Cpr$

3. Calculated by cells or bacteria number

Unit Definition: One unit defines as the change of absorbance at 460 nm by 0.01 per min per 10,000 bacteria or cells. $POD (U/10^4) = \Delta A \times V_{Total} \div (500 \times V_{Sample} \div V_{Sample \text{ Total}}) \div 0.01 \div T = 4 \times \Delta A$

4. Calculated by volume of liquid samples

Unit Definition: One unit defines as the change of absorbance at 460 nm by 0.01 per min per 1 mL Liquid Sample. $POD (U/mL) = \Delta A \times V_{Total} \div V_{Sample} \div 0.01 \div T = 2,000 \times \Delta A$

Where: V_{Total} : total reaction volume, 0.2 mL;

V_{Sample} : sample volume added, 0.01 mL;

$V_{Sample \text{ Total}}$: extract buffer added to samples, 1 mL;

W: sample weight, g; T: reaction time, 1 min;

Cpr: sample protein concentration, mg/mL;

500: Total number of bacteria or cells, 5×10^6 .

Disclaimer

1. The reagent is only used in the field of scientific research, not suitable for clinical diagnosis or other purposes.
2. For your safety and health, please wear a lab coat and disposable gloves.
3. This kit is not compared with similar kits from other manufacturers or products with different methods to detect the same object, so inconsistent test results cannot be ruled out.