Peroxidase Microplate Assay Kit

Cat #: orb390768 (manual)

Detection and Quantification of Peroxidase (POD) Activity in Urine, Serum, Plasma, Tissue extracts, Cell lysate, Cell culture media and Other biological fluids Samples.

For research use only. Not for diagnostic or therapeutic procedures.

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INTRODUCTION

Peroxidase (EC 1.11.1.7) is an enzyme found broadly in biological systems that utilizes hydrogen peroxide in the oxidation of various substrates. Peroxidases catalyze oxidation-reduction reactions and play an important role in protecting cell from oxidative injury.

The assay is initiated with the enzymatic catalysis of H2O2 by POD. The enzyme catalysated reaction products can be measured at a colorimetric readout at 470 nm.

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KIT COMPONENTS

Component	Volume	Storage
96-Well Microplate	1 plate	
Assay Buffer	30 ml x 4	4 °C
Reaction Buffer	5 ml x 1	4 °C
Substrate	10 ml x 1	4 °C, keep in dark
Dye Reagent	4 ml x 1	4 °C
Positive Control	Powder x 1	-20 °C
Technical Manual	1 Manual	

Note:

Positive Control: add 1 ml distilled water to dissolve before use, then take 30 µl positive control solution into 970 µl distilled water, mix.

MATERIALS REQUIRED BUT NOT PROVIDED

1. Microplate reader to read absorbance at 470 nm
2. Distilled water
3. Pipettor, multi-channel pipettor
4. Pipette tips
5. Mortar
6. Centrifuge
7. Timer
8. Ice

SAMPLE PREPARATION

1. For cell and bacteria samples

Collect cell or bacteria into centrifuge tube, discard the supernatant after centrifugation, add 1 ml Assay buffer for 5×10^6 cell or bacteria, sonicate (with power 20%, sonicate 3s, interval 10s, repeat 30 times) ; centrifuged at 8000g 4 °C for 20 minutes, take the supernatant into a new centrifuge tube and keep it on ice for detection.

2. For tissue samples

Weigh out 0.1 g tissue, homogenize with 1 ml Assay buffer on ice, centrifuged at 8000g 4 °C for 20 minutes, take the supernatant into a new centrifuge tube and keep it on ice for detection.

3. For serum or plasma samples

Detect directly.

ASSAY PROCEDURE

Warm the Substrate, Dye Reagent to room temperature before use.

Add following reagents into the microplate:

Reagent	Sample	Positive Control
Sample	10 µl	--
Positive Control	--	10 µl
Reaction Buffer	50 µl	50 µl
Substrate	100 µl	100 µl
Dye Reagent	40 µl	40 µl
Mix, measured at 470 nm and record the absorbance of 20th second and 140th second.		

CALCULATION

Unit Definition: one unit is defined as the OD value changed 0.01 per minute in the reaction system.

1. According to the protein concentration of sample

$$POD\ (U/mg) = (OD_{Sample\ (140S)} - OD_{Sample\ (20S))} \times V_{Total} / (V_{Sample} \times C_{Protein}) / T / 0.01$$

$$= 1000 \times (OD_{Sample\ (140S)} - OD_{Sample\ (20S))} / C_{Protein}$$

2. According to the weight of sample

$$POD\ (U/g) = (OD_{Sample\ (140S)} - OD_{Sample\ (20S))} \times V_{Total} / (W \times V_{Sample} / V_{Assay}) / T / 0.01$$

$$= 1000 \times (OD_{Sample\ (140S)} - OD_{Sample\ (20S))} / W$$

3. According to the quantity of cells or bacteria

$$POD\ (U/10^4) = (OD_{Sample\ (140S)} - OD_{Sample\ (20S))} \times V_{Total} / (N \times V_{Sample} / V_{Assay}) / T / 0.01$$

$$= 1000 \times (OD_{Sample\ (140S)} - OD_{Sample\ (20S))} / N$$

4. According to the volume of serum or plasma

$$POD\ (U/ml) = (OD_{Sample\ (140S)} - OD_{Sample\ (20S))} \times V_{Total} / V_{Sample} / T / 0.01$$

$$= 1000 \times (OD_{Sample\ (140S)} - OD_{Sample\ (20S))}$$

$C_{Protein}$: the protein concentration, mg/ml;

W: the weight of sample, g;

N: the quantity of cell or bacteria, $N \times 10^4$;

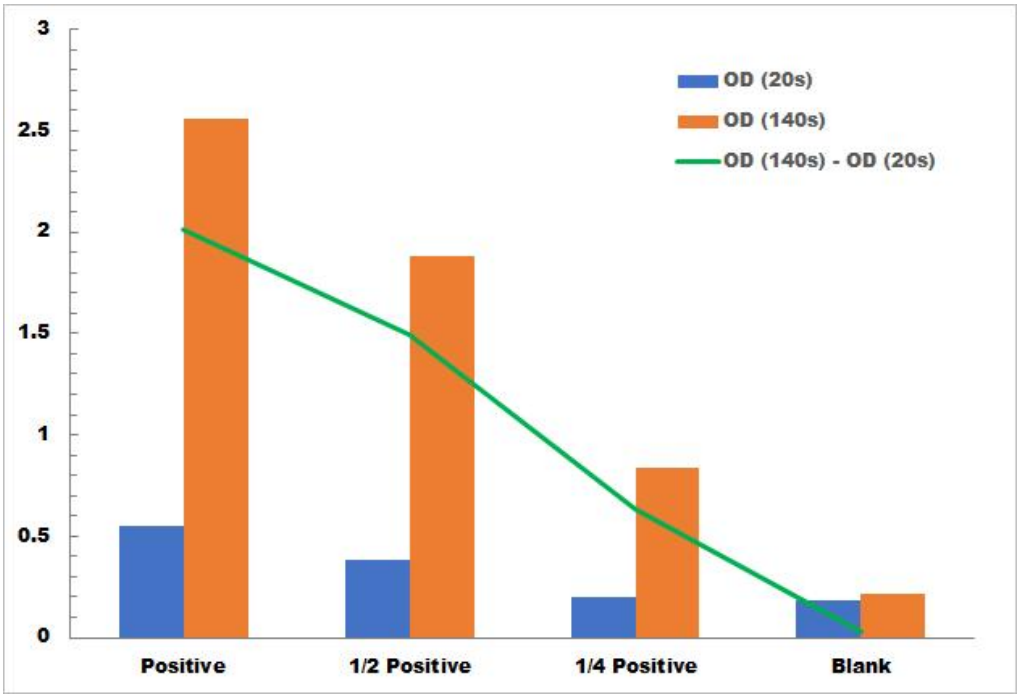
V_{Total}: the total volume of the enzymatic reaction, 0.2 ml;

V_{Sample}: the volume of sample, 0.01 ml;

V_{Assay}: the volume of Assay buffer in sample preparation, 1 ml;

T: the reaction time, 2 minutes.

TYPICAL DATA



Positive Control reaction in 96-well plate assay with decreasing the concentration