



Peroxidase Activity Colorimetric Microplate Assay Kit User Manual

Catalog # CAK1062

(Version 2.3E)

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

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

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I. 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.

Peroxidase Activity Colorimetric Microplate Assay Kit provides a simple and direct procedure for measuring peroxidase activity in a variety of samples. The assay is initiated with the enzymatic hydrolysis of H_2O_2 by peroxidase. The reaction product can react with the dye reagent, and measured at a colorimetric readout at 570 nm.

II.KIT COMPONENTS

Component	Volume	Storage
96-Well Microplate	1 plate	
Assay Buffer	30mlx 4	4 °C
Reaction Buffer	20 ml x 1	4 °C
Substrate	1 mlx 1	4 °C, keep in dark
Diluent	12 ml x 1	RT
Dye Reagent	Powder x 1	-20 °C, keep in dark
Standard	Powder x 1	4 °C, keep in dark
Positive Control	Powderx 1	-20 °C
Technical Manual	1 Manual	

III. MATERIALS REQUIRED BUT NOT PROVIDED

1. Microplate reader to read absorbance at 570 nm
2. Distilled water
3. Pipettor, multi-channel pipettor
4. Pipette tips
5. Mortar
6. Centrifuge
7. Timer
8. Ice

IV. REAGENT PREPARATION

Standard: Warm the Diluent to RT and briefly centrifuge the Standard prior to opening. Dissolve Standard in 10 ml Diluent and mix for half an hour to generate 1 mmol/L of standard stock solution. Keep in dark and store at 4 °C for 2-3 weeks or -20°C for 2-3 months after reconstitution. Then dilute to 0.2 mmol/L standard top solution by adding 200 µl stock solution into 800 µl distilled water. Perform 2-fold serial dilutions of the top standard solution using distilled water to make the standard curve. The concentration of standard curve could be 200/100/50/25/12.5/6.25/3.12 µmol/L.

Dye Reagent: Warm the Diluent to RT, then add 1 ml Diluent to dissolve. Protect from light and moisture and store at 4 °C for 1 week or -20°C for 2 months after reconstitution.

Positive Control: Briefly centrifuge prior to opening. Add 1 ml Reaction Buffer to dissolve before use; then add 25 µl into 975 µl Reaction Buffer. Store at -80°C for 1 month after reconstitution.

Note: Divide into small aliquots to avoid repeated freeze-thaw cycles.

V. 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 liquid samples

Detect directly.

VI. ASSAY PROCEDURE

Warm all reagents to room temperature before use.

Add following reagents into the microplate.

Reagent*	Sample**	Control	Standard	Blank	Positive Control
Reaction Buffer	170 μ l	170 μ l	--	--	170 μ l
Sample	10 μ l		--	--	--
Positive Control	--	--	--	--	10 μ l
Distilled water	--	10 μ l	--	200 μ l	--
Standard	--		200 μ l	--	--
Dye Reagent	10 μ l	10 μ l	--	--	10 μ l
Mix.					
Substrate	10 μ l	10 μ l	--	--	10 μ l
Mix, put it in the oven, 37 °C for 2 minutes, measured at 570 nm and record the absorbance.					

Note:

*Reagents must be added sequentially and should not be premixed prior to addition.

**For unknown samples, we recommend doing a pilot experiment & testing several doses to ensure the readings are within the standard curve range. If the enzyme activity is lower, please add more samples into the reaction system; or increase the reaction time; if the enzyme activity is higher, please dilute the sample, or decrease the reaction time.

**For colored samples, we recommend setting a parallel sample background control well with same volume of sample only. Other reagents were replaced by distilled water to the same total volume. Subtract the OD value of the sample background control from the OD value of the sample to correct for interference from the sample's own color.

VII. CALCULATION

Unit Definition: one unit is defined as the enzyme will catalyze the formation of 1 μ mole resorufin per min.

1. According to the slope of the standard curve

$$\text{Activity} = \frac{(\text{OD}_{\text{Sample}} - \text{OD}_{\text{Control}}) - \text{Intercept}}{\text{Slope} \times T} \times \frac{V_{\text{Standard}}}{V_{\text{Sample}}} \times n (\text{U/mL})$$

2. According to one point of the standard OD value and concentration

2.1 According to the protein concentration of sample

$$\text{Activity} = \frac{(C_{\text{Standard}} \times V_{\text{Standard}}) \times (\text{OD}_{\text{Sample}} - \text{OD}_{\text{Control}})}{(\text{OD}_{\text{Standard}} - \text{OD}_{\text{Blank}}) \times V_{\text{Sample}} \times C_{\text{Protein}} \times T} (\text{U/mg})$$

2.2 According to the quantity of cells or bacteria

$$\text{Activity} = \frac{(C_{\text{Standard}} \times V_{\text{Standard}}) \times (\text{OD}_{\text{Sample}} - \text{OD}_{\text{Control}})}{(\text{OD}_{\text{Standard}} - \text{OD}_{\text{Blank}}) \times N \times (V_{\text{Sample}} / V_{\text{Assay}}) \times T} (\text{U}/10^4)$$

2.3 According to the weight of sample

$$\text{Activity} = \frac{(C_{\text{Standard}} \times V_{\text{Standard}}) \times (\text{OD}_{\text{Sample}} - \text{OD}_{\text{Control}})}{(\text{OD}_{\text{Standard}} - \text{OD}_{\text{Blank}}) \times W \times (V_{\text{Sample}} / V_{\text{Assay}}) \times T} (\text{U/g})$$

2.4 According to the volume of sample

$$\text{Activity} = \frac{(C_{\text{Standard}} \times V_{\text{Standard}}) \times (\text{OD}_{\text{Sample}} - \text{OD}_{\text{Control}})}{(\text{OD}_{\text{Standard}} - \text{OD}_{\text{Blank}}) \times V_{\text{Sample}} \times T} (\text{U/mL})$$

Slope: the absorbance slope of standard curve

n: the dilution factor

C_{Protein} : the protein concentration of sample, mg/mL

W: the weight of total sample, g

N: the quantity of total cell or bacteria sample, 10^4

C_{Standard} : the concentration of standard, μ mol/L

V_{Standard} : the volume of standard in assay procedure, mL

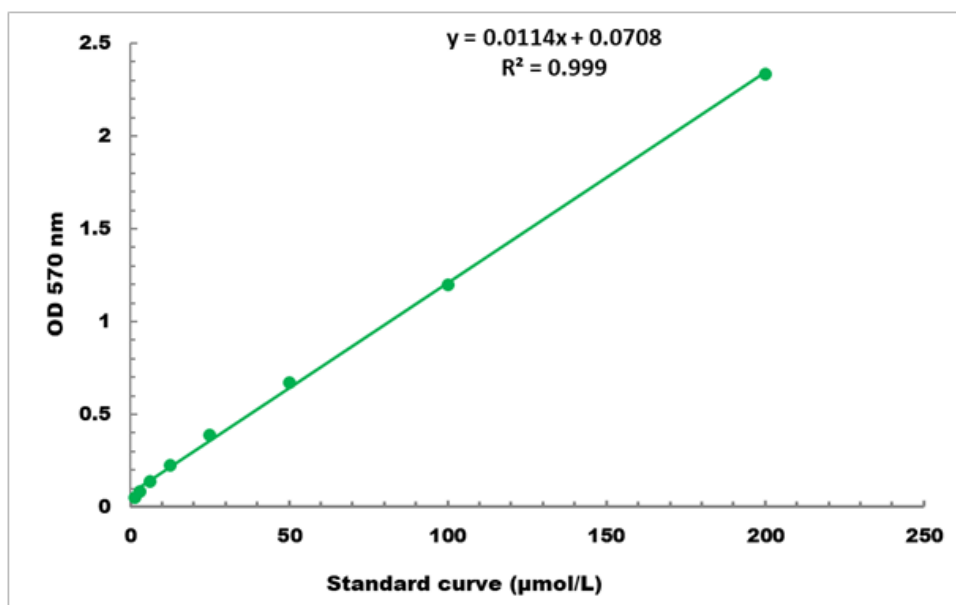
V_{Sample} : the volume of sample in assay procedure, mL

V_{Assay} : the volume of Assay Buffer in sample preparation, mL

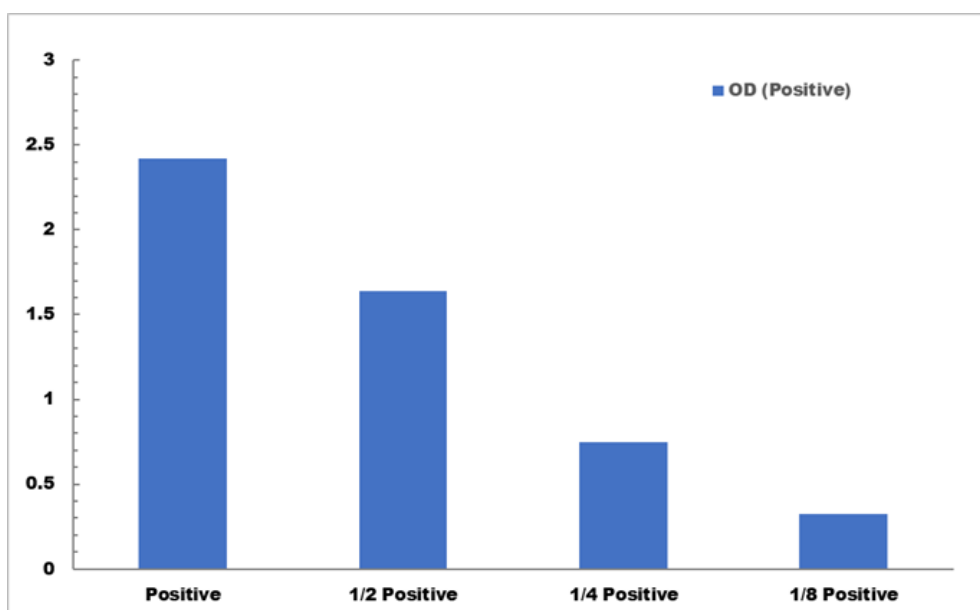
T: the reaction time, minute

VIII. TYPICAL DATA

The standard curve is for demonstration only. A standard curve must be run with each assay.



Detection Range: 2 μmol/L - 200 μmol/L



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

