Peroxidase Activity

Fluorometric Microplate Assay Kit

User Manual

Catalog # CAK8017

(Version 1.1A)

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.

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 Fluorometric 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 probe, which can be detected fluorometrically (Ex/Em 535/587).

II. KIT COMPONENTS

Component	Volume	Storage
96-Well Microplate	1 plate	
Assay Buffer	30 ml x 4	4 °C
Reaction Buffer	20 ml x 1	4 °C
Substrate	1 ml x 1	4 °C, keep in dark
Diluent	12 ml x 1	RT
Probe	Powder x 1	-20 °C, keep in dark
Standard	Powder x 1	4 °C, keep in dark
Positive Control	Powder x 1	-20 °C
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Note:

Dye Reagent: Warm the Diluent to RT, then add 1 ml Diluent to dissolve. Store at -20 °C, protect from light and moisture. Used within 1 month.

Standard: Warm the Diluent to RT, add 10 ml Diluent to dissolve, mix for half an hour; then add 20 µl into 980 µl distilled water, mix. The concentration will be 20 µmol/L. Store at -20°C for 1 month.

Positive Control: add 1 ml Reaction Buffer to dissolve before use; then add 2 µl into 998 µl Reaction Buffer. Store at -80 °C for 1 month after reconstitution.

III. MATERIALS REQUIRED BUT NOT PROVIDED

1. Fluorescence microplate reader to read fluorescence at Ex/Em = 535/587
2. Distilled water
3. Pipettor, multi-channel pipettor
4. Pipette tips
5. Mortar
6. Centrifuge
7. Timer
8. Ice

IV. 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.

V. ASSAY PROCEDURE

Warm all reagent to room temperature before use.

Add following reagents into the microplate.

Reagent	Sample	Control	Standard	Blank	Positive Control
Reaction Buffer	170 µl	170 µl	--	--	170 µl
Sample	10 µl		--	--	--
Positive Control	--	--	--	--	10 µl
Distilled water	--	10 µl	--	200 µl	--
Standard	--		200 µl	--	--
Dye Reagent	10 µl	10 µl	--	--	10 µl
Mix.					
Substrate	10 µl	10 µl	--	--	10 µl
Mix, put it in the oven, 37 °C for 2 minutes, protected from light, record fluorescence measured at Ex/Em = 535/587 nm.					

Note:

1) Perform 2-fold serial dilutions of the top standards to make the standard curve.

2) 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.

3) Reagents must be added step by step, can not be mixed and added together.

VI. CALCULATION

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

1. According to the protein concentration of sample

$$POD\ (U/mg) = (C_{Standard} \times V_{Standard}) \times (OD_{Sample} - OD_{Control}) / (OD_{Standard} - OD_{Blank}) / (V_{Sample} \times C_{Protein}) / T$$

$$= 0.2 \times (OD_{Sample} - OD_{Control}) / (OD_{Standard} - OD_{Blank}) / C_{Protein}$$

2. According to the volume of sample

$$POD\ (U/ml) = (C_{Standard} \times V_{Standard}) \times (OD_{Sample} - OD_{Control}) / (OD_{Standard} - OD_{Blank}) / V_{Sample} / T$$

$$= 0.2 \times (OD_{Sample} - OD_{Control}) / (OD_{Standard} - OD_{Blank})$$

3. According to the weight of sample

$$POD\ (U/g) = (C_{Standard} \times V_{Standard}) \times (OD_{Sample} - OD_{Control}) / (OD_{Standard} - OD_{Blank}) / (V_{Sample} \times W / V_{Assay}) / T$$

$$= 0.2 \times (OD_{Sample} - OD_{Control}) / (OD_{Standard} - OD_{Blank}) / W$$

4. According to the quantity of cells or bacteria

$$POD\ (U/10^4) = (C_{Standard} \times V_{Sample}) \times (OD_{Sample} - OD_{Control}) / (OD_{Standard} - OD_{Blank}) / (V_{Sample} \times N / V_{Assay}) / T$$

$$= 0.2 \times (OD_{Sample} - OD_{Control}) / (OD_{Standard} - OD_{Blank}) / N$$

$C_{Standard}$: the Standard concentration, 20 μmol/L = 0.02 μmol/ml

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

W: the weight of sample, g

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

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

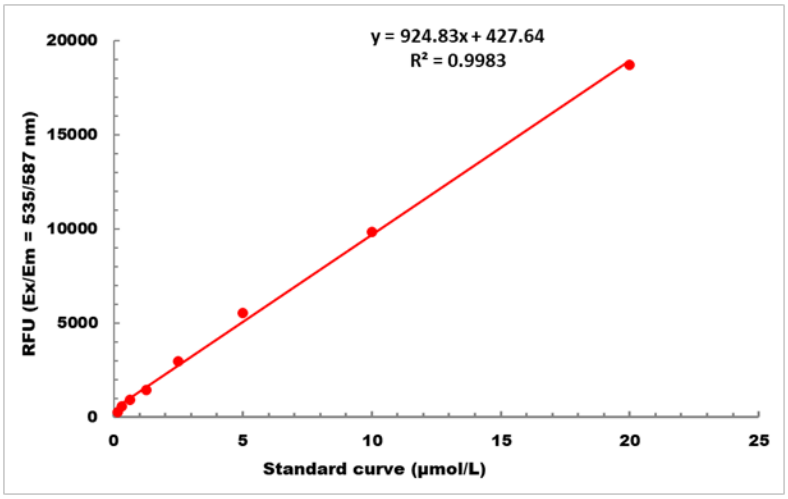
$V_{Standard}$: the volume of standard, 0.2 ml

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

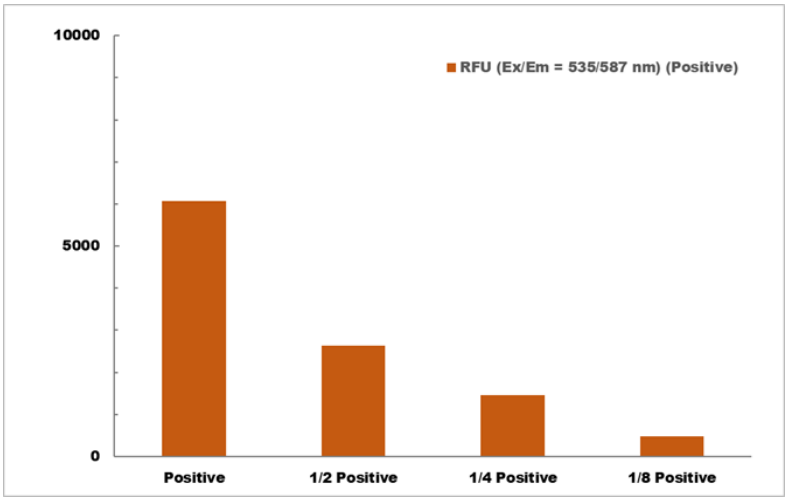
T: the reaction time, 2 minutes

VII. TYPICAL DATA

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



Detection Range: 0.2 μmol/L - 20 μmol/L



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

VIII. TECHNICAL SUPPORT

For troubleshooting, information or assistance, please go online to www.cohesionbio.com or contact us at techsupport@cohesionbio.com

IX. NOTES