Peroxidase Activity

Fluorometric Microplate Assay Kit

User Manual

Catalog # CAK8017

(Version 1.2A)

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.

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 ActivityFluorometric 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	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
Probe	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. 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. REAGENT PREPARATION

Standard:Warm the Diluent to RT and briefly centrifuge prior to opening.Dissolvein1 ml Diluentto generate 1 mmol/L of standardstock solution.Keep in dark and store at 4 °C for 2-3 weeks or -20°C for 6months after reconstitution.Then dilute to 20 µmol/Lstandard top solutionby adding 20 µlstock solution into 980 µldistilled water. Perform 2-fold serial dilutions of the top standardsolution using distilled waterto make the standard curve. The concentration of standard curve could be 20/10/5/2.5/1.25/0.625/0.312µmol/L.

Dye Reagent:Warm the Diluent to RT and briefly centrifuge prior to opening.Add 1 ml Diluent to dissolve. Store at -20 °C, protect from light and moisture. Used within 1 month.

Positive Control: Briefly centrifuge prior to opening. Add 1 ml Reaction Buffer to dissolve before use; then add 2 µl into 998 µl Reaction Buffer.Store at -80°C for 1 monthafter 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 mlAssay 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 20minutes, take the supernatant into a new centrifuge tube and keep it on ice for detection.

2.For tissue samples

Weighout 0.1 g tissue, homogenize with 1 mlAssay Buffer on ice, centrifuged at 8000g 4°C for 20minutes, take the supernatant into a new centrifuge tube and keep it on ice for detection.

3.For liquid samples

Detect directly.

VI. ASSAY PROCEDURE

Warmall reagentto room temperature before use.

Add following reagents intothe microplate.

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

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.

VII. CALCULATION

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

1. According to the slope of the standard curve

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

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

2.1 According to the protein concentration of sample

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

2.2According to the quantity of cells or bacteria

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

2.3 According to the weight of sample

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

2.4 According to the volume of sample

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

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, μmol/mL

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

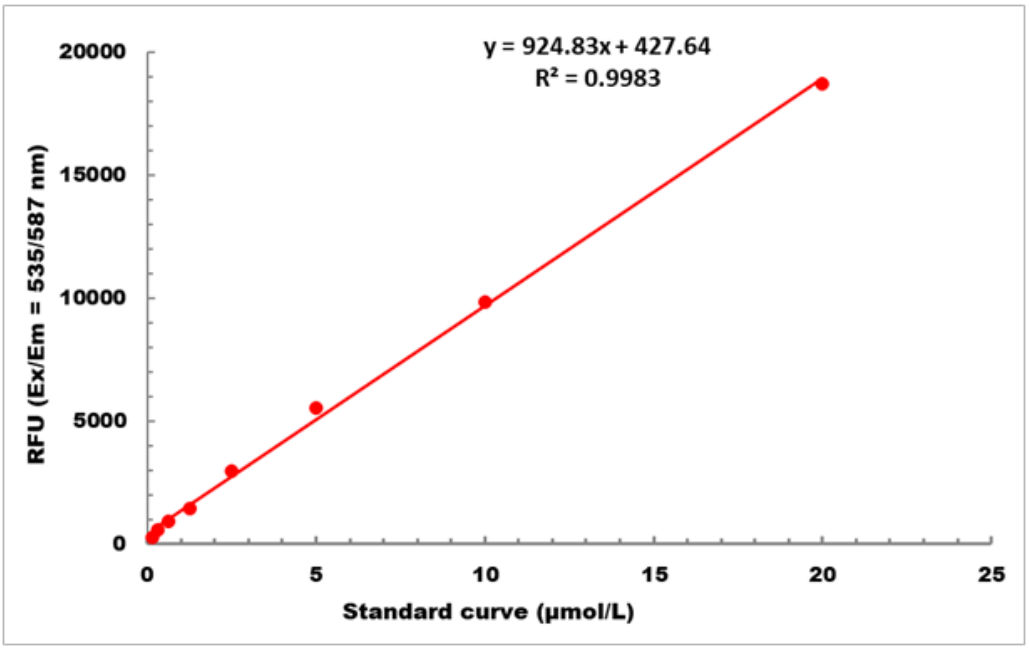
V_{Sample}: the volume of sample in assay procedure, mL

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

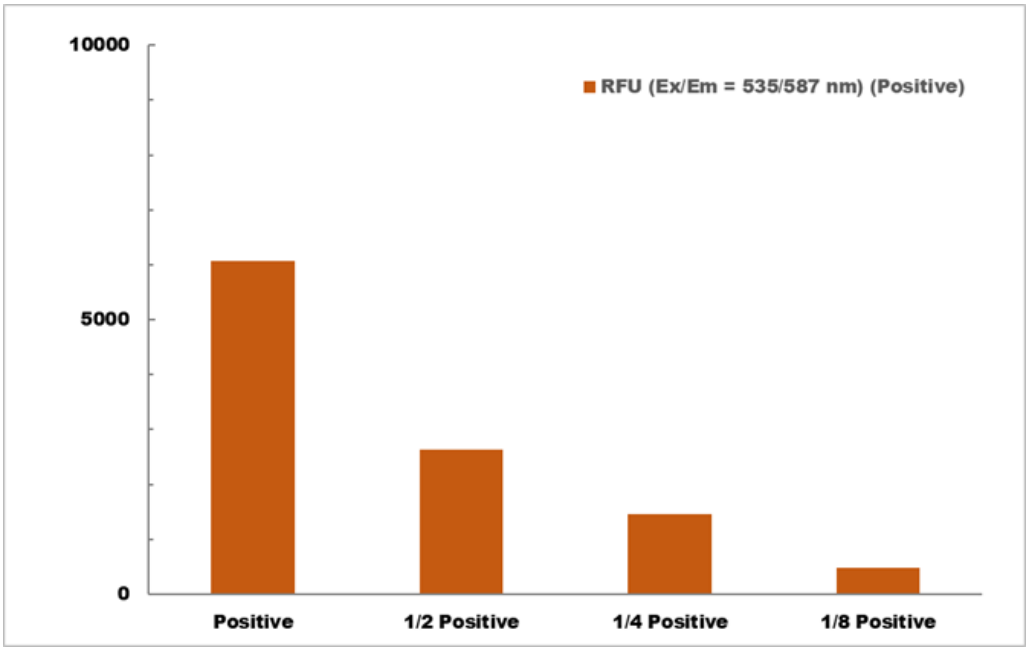
T: the reaction time, minute

VIII. TYPICAL DATA

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



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



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