Glutathione Peroxidase Activity

Colorimetric Microplate Assay Kit

User Manual

Catalog # CAK1149

(Version 2.5C)

Detection and Quantification of Glutathione Peroxidase(GPX) ActivityinSerum, Plasma, Other biological fluids, Tissue extracts, Cell lysate, Cell culture mediaSamples.

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

I. INTRODUCTION

Glutathione Peroxidase (EC 1.11.1.9) family of enzymes play important roles in the protection of organisms from oxidative damage. GPX converts reduced glutathione (GSH) to oxidized glutathione (GSSG) while reducing lipid hydroperoxides to their corresponding alcohols or free hydrogen peroxide to water. Several isozymes have been found in different cellular locations and with different substrate specificity. Low levels of GPX have been correlated with free radical related disorders.

Glutathione Peroxidase Activity Colorimetric Microplate Assay Kit is a sensitive assay for determining Glutathione Peroxidase activity in various samples. GPX reduces Hydroperoxide while oxidizing GSH to GSSG. The generatedGSSG is reduced to GSH with consumption of NADPH. The decrease of NADPHis proportional to GPX activity.

II.KIT COMPONENTS

Component	Volume	Storage
96-Well Microplate	1 plate	
Assay Buffer	30mlx 4	4 °C
Reaction Buffer	6 ml x 1	4 °C
Substrate I	Powder x 1	4 °C, keep in dark
Substrate II (4000X)	0.2 ml x 1	4 °C, keep in dark
Substrate Diluent	12 mlx 1	4 °C
Enzyme	Powder x 1	-20 °C, keep in dark
Enzyme Diluent	1 mlx 1	4 °C
Dye ReagentA	Powder x 1	4 °C, keep in dark
Dye Reagent B	1 ml x 1	4 °C, keep in dark
Standard	Powder x 1	4 °C, keep in dark
Positive Control	0.05 ml x 1	-20 °C
Technical Manual	1 Manual	

III. MATERIALS REQUIRED BUT NOT PROVIDED

1. Microplate reader to read absorbance at 450 nm
2. Distilled water
3. Pipettor, multi-channel pipettor
4. Pipette tips
5. Mortar
6. Centrifuge
7. Timer
8. Water bath

IV. REAGENT PREPARATION

Standard:Briefly centrifuge prior to opening. Dissolvein1 ml distilled waterto generate 2mmol/L of stockstandardsolution. Then add 0.05 ml into 0.45 ml distilled water to generate 200 µmol/L as the top standardsolution. Then perform 2-fold serial dilutions of the top solution using distilled waterto make the standard curve. The concentration of standard curve could be 200/100/50/25/12.5/6.25µmol/L.Store at -20 °C for 1 weekor 4°C for 1 day.

Substrate I:Briefly centrifuge prior to opening. Add 1 ml Substrate Diluent to dissolve before use.Store at -20 °C for 2weeksor 4°C for 1 day.

Substrate II:Briefly centrifuge prior to opening. Transfer 10µlSubstrate II(4000X) into 390 µlSubstrate Diluent and mix well to generate Substrate II (100X). Thenadd10µl Substrate II(100X) into990µl Substrate Diluent to generate Substrate II (1X). Keep the diluted solutions (100X and 1X) in dark and usethem within 1 hour.

Enzyme: Briefly centrifuge prior to opening. Add 1 ml Enzyme Diluentto dissolve before use.Store at -80 °C for 1 monthafter reconstitution.

Dye Reagent A: Add 9 mldistilled water to dissolve before use, mix, store at 4°C for 1 monthafter reconstitution.

Dye ReagentWorking Solution: Mix Dye Reagent A and Dye ReagentB at ratio of 9:1 to generate the Dye Reagentworking solution (e.g. 900uL Dye Reagent A and 100uL Dye ReagentB for 10 tests). Keep in dark and store at 4°C for 1 month.

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 10 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 mlAssay Buffer on ice, centrifuged at 8000g 4°C for 10 minutes, take the supernatant into a new centrifuge tube and keep it on ice for detection.

3.For serum, plasma or other biological fluidssamples

Detect directly.

VI. ASSAY PROCEDURE

Add following reagents in the microcentrifuge tube:

Reagent*	Sample**	Control	Positive Control	Standard	Blank
Reaction Buffer	60 µl	60 µl	60 µl	--	--
Sample	10 µl	--	--	--	--
Distilled water	--	10 µl	--	--	100 µl
Positive Control	--	--	10 µl	--	--
Standard	--	--	--	100 µl	--
Substrate I	10 µl	10 µl	10 µl	--	--
Enzyme	10 µl	10 µl	10 µl	--	--
Substrate II (1X)	10 µl	10 µl	10 µl	--	--
Dye ReagentWorking Solution	100 µl	100 µl	100 µl	100 µl	100 µl
Mix andput in the oven at 37 °C for 10 minutes, record absorbance measured at 450 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.

**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 of GPX activity is defined as the enzyme decomposes1 μmol of NAPDH to $NADP^+$ per minute.

1. According to the slope of the standard curve

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

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

2.1According to the quantity of cells

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

2.2 According to the weight of sample

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

2.3 According to the volume of sample

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

Slope: the absorbance slope of standard curve

n: the dilution factor

W: the weight of total sample, g

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

$C_{Standard}$: the concentration of standard, μmol/mL

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

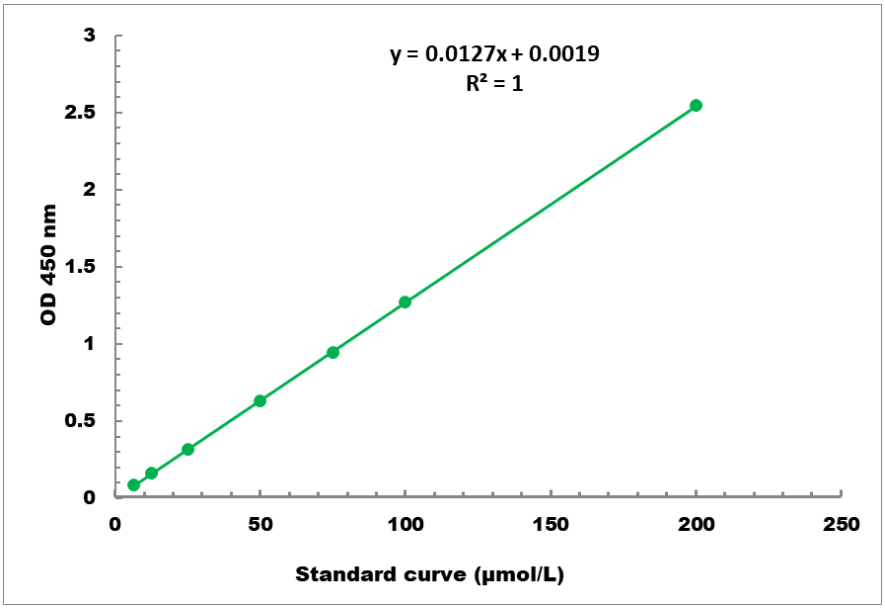
V_{Sample}: the volume of sample in assay procedure, mL

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

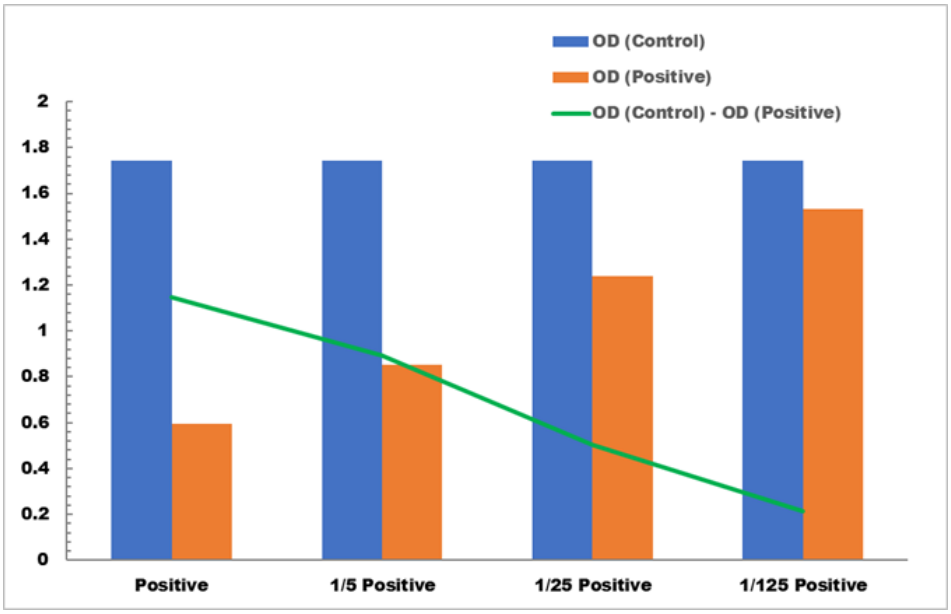
T: the reaction time, minute

VIII. TYPICAL DATA

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



Detection Range: 2μmol/L-200μmol/L



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