Glutathione Peroxidase

Microplate Assay Kit

User Manual

Catalog # CAK1149

(Version 1.3A)

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

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 Microplate Assay Kit is a sensitive assay for determining Glutathione Peroxidase activity in various samples. GPX reduces Hydroperoxide while oxidizing GSH to GSSG. GSH may react with DTNB. The decrease of GSH is proportional to GPX activity.

II. KIT COMPONENTS

Component	Volume	Storage
96-Well Microplate	1 plate	
Assay Buffer	30 ml x 4	4 °C
Substrate I	Powder x 1	4 °C
Substrate II	1 ml x 1	4 °C
Reaction Buffer	10 ml x 1	4 °C
Stop Solution	15 ml x 1	4 °C
Dye Reagent	Powder x 1	4 °C
Standard	Powder x 1	4 °C
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Note:

Substrate I: add 2 ml distilled water to dissolve before use.

Dye Reagent: add 2 ml distilled water to dissolve before use.

Standard: add 1 ml distilled water to dissolve, then add 500 µl Standard into 500 µl distilled water, the concentration will be 5 mmol/L.

III. MATERIALS REQUIRED BUT NOT PROVIDED

1. Microplate reader to read absorbance at 423 nm
2. Distilled water
3. Pipettor, multi-channel pipettor
4. Pipette tips
5. Mortar
6. Centrifuge
7. Timer
8. Water bath

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 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 ml Assay 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 fluids samples

Detect directly.

V. ASSAY PROCEDURE

Add following reagents in the microcentrifuge tube:

Reagent	Sample	Control	Standard	Blank
Substrate I	20 µl	20 µl	--	--
Sample	20 µl	--	--	--
Distilled water	--	20 µl	30 µl	50 µl
Shake and mix, put it in water bath of 37 °C for 30 minutes.				
Substrate II	10 µl	10 µl	--	--
Shake and mix, put it in water bath of 37 °C for 3 minutes.				
Standard	--	--	20 µl	--
Stop Solution	150 µl	150 µl	150 µl	150 µl
Shake and mix, centrifuged at 5000g for 10 minutes. Then add the supernatant into the microplate.				
Supernatant	80 µl	80 µl	80 µl	80 µl
Reaction Buffer	100 µl	100 µl	100 µl	100 µl
Dye Reagent	20 µl	20 µl	20 µl	20 µl
Record absorbance measured at 423 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 sample 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 of GPX activity is defined as the enzyme decomposes 1 μmol of GSH per minute.

1. According to the protein concentration of sample

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

$$= 1.666 \times (OD_{Control} - OD_{Sample}) / (OD_{Standard} - OD_{Blank}) / C_{Protein}$$

2. According to the weight of sample

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

$$= 1.666 \times (OD_{Control} - OD_{Sample}) / (OD_{Standard} - OD_{Blank}) / W$$

3. According to the volume of sample

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

$$= 1.666 \times (OD_{Control} - OD_{Sample}) / (OD_{Standard} - OD_{Blank})$$

$C_{Standard}$: the standard concentration, 5 mmol/L = 5 μmol/ml;

$V_{Standard}$: the volume of standard, 0.02 ml;

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

W: the weight of sample, g;

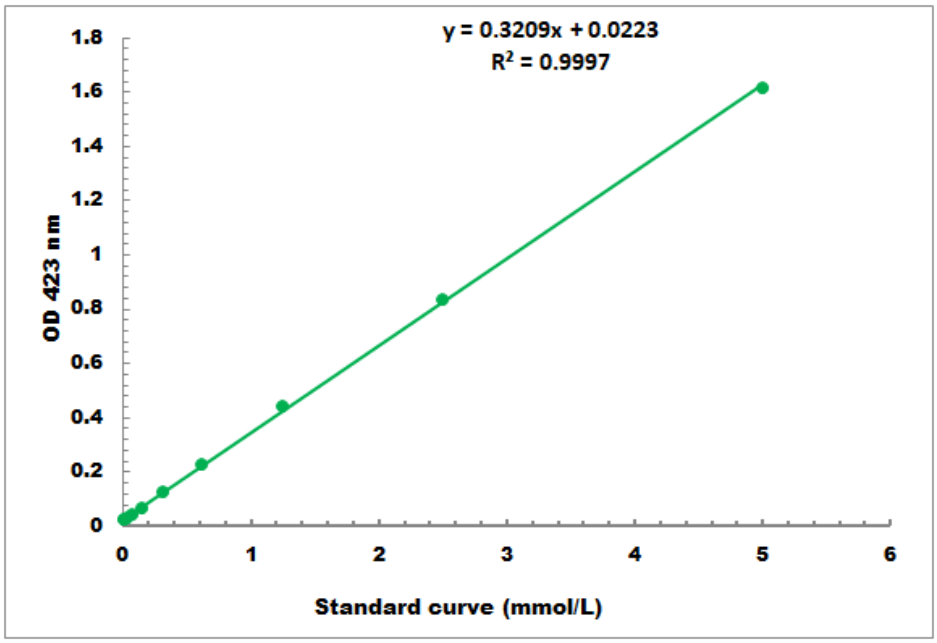
V_{Sample}: the volume of sample, 0.02 ml;

V_{Assay}: the volume of Assay Buffer, 1 ml;

T: the reaction time, 3 minutes.

VII. TYPICAL DATA

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



Detection Range: 0.01 mmol/L - 5 mmol/L

VIII. TECHNICAL SUPPORT

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

IX. NOTES