

## **Glutathione Reductase**

### **Microplate Assay Kit**

**Cat #: orb390749 (manual)**

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

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

## INTRODUCTION

Glutathione reductase (GR, EC 1.6.4.2) is a flavoprotein that catalyzes the NADPH-dependent reduction of oxidized glutathione (GSSG) to glutathione (GSH). This enzyme is essential for the GSH redox cycle which maintains adequate levels of reduced cellular GSH. A high GSH/GSSG ratio is essential for protection against oxidative stress.

Glutathione Reductase Microplate Assay Kit measures GR activity by measuring the rate of NADPH oxidation. The oxidation of NADPH to NADP<sup>+</sup> is accompanied by a decrease in absorbance at 340 nm. Since GR is present at rate limiting concentrations, the rate of decrease in the A<sub>340</sub> is directly proportional to the GR activity in the sample.

**KIT COMPONENTS**

Component	Volume	Storage
96-Well Microplate	1 plate	
Assay Buffer	30 ml x 4	4 °C
Substrate	Powder x 1	-20 °C
Standard	Powder x 1	-20 °C
Positive Control	10 µl	4 °C
Technical Manual	1 Manual	

**Note:**

**Substrate:** add 19 ml Assay Buffer to dissolve before use.

**Standard:** add 1 ml distilled water to dissolve before use; then add 0.2 ml into 0.8 ml distilled water, the concentration will be 400 µmol/L.

**Positive Control:** add 90 µl distilled water to dissolve before use.

**MATERIALS REQUIRED BUT NOT PROVIDED**

1. Microplate reader to read absorbance at 340 nm
2. Distilled water
3. Pipettor, multi-channel pipettor
4. Pipette tips
5. Mortar
6. Centrifuge
7. Timer
8. Ice

## 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  $5 \times 10^6$  cell or bacteria, sonicate (with power 20%, sonicate 3s, interval 10s, repeat 30 times); centrifuged at 8,000g 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 8,000g 4 °C for 20 minutes, take the supernatant into a new centrifuge tube and keep it on ice for detection.

### 3. For serum or plasma samples

Detect directly.

**ASSAY PROCEDURE**

Warm all reagents to room temperature before use.

Add following reagents in the microplate:

Reagent	Sample	Standard	Blank	Positive Control
Standard	--	200 $\mu$ l	--	--
Distilled water	--	--	200 $\mu$ l	--
Substrate	190 $\mu$ l	--	--	190 $\mu$ l
Sample	10 $\mu$ l	--	--	--
Positive Control	--	--	--	10 $\mu$ l

Mix, measured at 340 nm and record the absorbance of 10th second and 130th second.

**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.

## CALCULATION

**Unit Definition:** One unit of GR activity is defined as the enzyme that reduces 1 nmol of NADPH per minute.

1. According to the protein concentration of sample

$$\begin{aligned} \text{GR (U/mg)} &= (C_{\text{Standard}} \times V_{\text{Standard}}) \times (\text{OD}_{\text{Sample (10S)}} - \text{OD}_{\text{Sample (130S)}}) / (\text{OD}_{\text{Standard}} - \text{OD}_{\text{Blank}}) / (V_{\text{Sample}} \times C_{\text{Protein}}) \\ & / T \\ &= 4000 \times (\text{OD}_{\text{Sample (10S)}} - \text{OD}_{\text{Sample (130S)}}) / (\text{OD}_{\text{Standard}} - \text{OD}_{\text{Blank}}) / C_{\text{Protein}} \end{aligned}$$

2. According to the weight of sample

$$\begin{aligned} \text{GR (U/g)} &= (C_{\text{Standard}} \times V_{\text{Standard}}) \times (\text{OD}_{\text{Sample (10S)}} - \text{OD}_{\text{Sample (130S)}}) / (\text{OD}_{\text{Standard}} - \text{OD}_{\text{Blank}}) / (V_{\text{Sample}} \times W / \\ & V_{\text{Assay}}) / T \\ &= 4000 \times (\text{OD}_{\text{Sample (10S)}} - \text{OD}_{\text{Sample (130S)}}) / (\text{OD}_{\text{Standard}} - \text{OD}_{\text{Blank}}) / W \end{aligned}$$

3. According to the quantity of cells or bacteria

$$\begin{aligned} \text{GR (U/10}^4\text{)} &= (C_{\text{Standard}} \times V_{\text{Standard}}) \times (\text{OD}_{\text{Sample (10S)}} - \text{OD}_{\text{Sample (130S)}}) / (\text{OD}_{\text{Standard}} - \text{OD}_{\text{Blank}}) / (V_{\text{Sample}} \times N / \\ & V_{\text{Assay}}) / T \\ &= 4000 \times (\text{OD}_{\text{Sample (10S)}} - \text{OD}_{\text{Sample (130S)}}) / (\text{OD}_{\text{Standard}} - \text{OD}_{\text{Blank}}) / N \end{aligned}$$

4. According to the volume of sample

$$\begin{aligned} \text{GR (U/ml)} &= (C_{\text{Standard}} \times V_{\text{Standard}}) \times (\text{OD}_{\text{Sample (10S)}} - \text{OD}_{\text{Sample (130S)}}) / (\text{OD}_{\text{Standard}} - \text{OD}_{\text{Blank}}) / V_{\text{Sample}} / T \\ &= 4000 \times (\text{OD}_{\text{Sample (10S)}} - \text{OD}_{\text{Sample (130S)}}) / (\text{OD}_{\text{Standard}} - \text{OD}_{\text{Blank}}) \end{aligned}$$

$C_{\text{Standard}}$ : the standard concentration, 400  $\mu\text{mol/L} = 400 \text{ nmol/ml}$ ;

$V_{\text{Standard}}$ : the volume of standard, 200  $\mu\text{l} = 0.2 \text{ ml}$ ;

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

W: the weight of sample, g;

N: the quantity of cell or bacteria,  $N \times 10^4$ ;

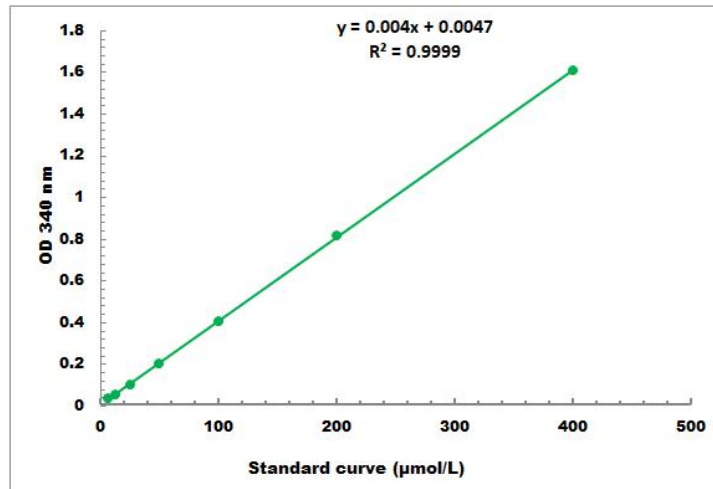
$V_{\text{Sample}}$ : the volume of sample, 0.01 ml;

$V_{\text{Assay}}$ : the volume of Assay buffer, 1 ml;

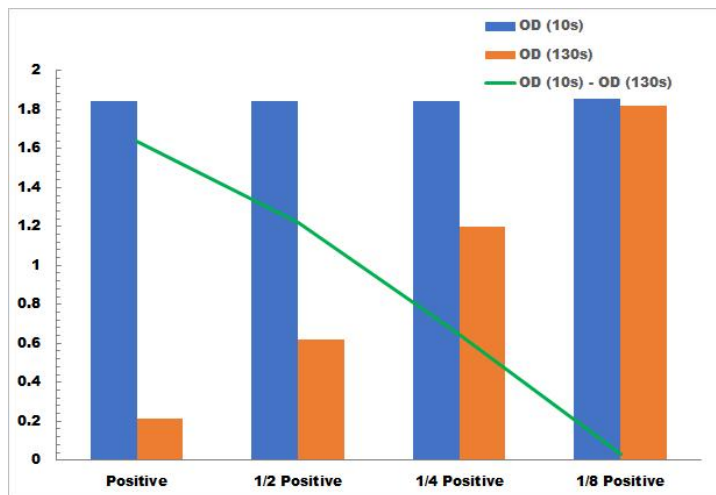
T: the reaction time, 2 minutes.

## TYPICAL DATA

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



Detection Range: 4 µmol/L - 400 µmol/L



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