Glutathione Reductase Microplate Assay Kit

Basic information:

Catalog No.: UAK1043 **Size:** 100 Assays

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

I. 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+ is accompanied by a decrease in absorbance at 340 nm. Since GR is present at rate limiting concentrations, the rate of decrease in the A340 is directly proportional to the GR activity in the sample.

II. KIT COMPONENTS

Component	Volume	Storage
96-Well Microplate	1 plate	
Assay Buffer	30 ml x 4	4 °C
Substrate	Powder x 1	4 °C
Standard	Powder x 1	-20 °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.*

III. MATERIALS REQUIRED BUT NOT PROVIDED

1. Microplate reader to read absorbance at 340 nm

2. Distilled water

3. 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%, sonication 3s, intervation 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.

V. ASSAY PROCEDURE

Warm all reagents to room temperature before use.

Add following reagents in the microplate:

Reagent	Sample	Standard	Blank
Standard	--	200 µl	--

Distilled water	--	--	200 μl
Substrate	190 μl	--	--
Sample	10 μl	--	--
Mix, measured at 340 nm and record the absorbance of 10th second and 130th second.			

***Note**: if the enzyme activity is lower, please add more sample into the reaction system; or increase the reaction time.*

VI. 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

$$GR\ (U/mg) = (C_{Standard} \times V_{Standard}) \times (OD_{Sample(10S)} - OD_{Sample(130S)}) / (OD_{Standard} - OD_{Blank}) / (V_{Sample} \times C_{Protein}) / T$$

$$= 4000 \times (OD_{Sample(10S)} - OD_{Sample(130S)}) / (OD_{Standard} - OD_{Blank}) / C_{Protein}$$

2. According to the weight of sample

$$GR\ (U/g) = (C_{Standard} \times V_{Standard}) \times (OD_{Sample(10S)} - OD_{Sample(130S)}) / (OD_{Standard} - OD_{Blank}) / (V_{Sample} \times W / V_{Assay}) / T$$

$$= 4000 \times (OD_{Sample(10S)} - OD_{Sample(130S)}) / (OD_{Standard} - OD_{Blank}) / W$$

3. According to the quantity of cells or bacteria

$$GR\ (U/10^4) = (C_{Standard} \times V_{Standard}) \times (OD_{Sample(10S)} - OD_{Sample(130S)}) / (OD_{Standard} - OD_{Blank}) / (V_{Sample} \times N / V_{Assay}) / T$$

$$= 4000 \times (OD_{Sample(10S)} - OD_{Sample(130S)}) / (OD_{Standard} - OD_{Blank}) / N$$

4. According to the volume of sample

$$GR\ (U/ml) = (C_{Standard} \times V_{Standard}) \times (OD_{Sample(10S)} - OD_{Sample(130S)}) / (OD_{Standard} - OD_{Blank}) / V_{Sample} / T$$

$$= 4000 \times (OD_{Sample(10S)} - OD_{Sample(130S)}) / (OD_{Standard} - OD_{Blank})$$

$C_{Standard}$: the standard concentration, 400 μmol/L = 400 nmol/ml;

$V_{Standard}$: the volume of standard, 200 μl = 0.2 ml;

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

W: the weight of sample, g;

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

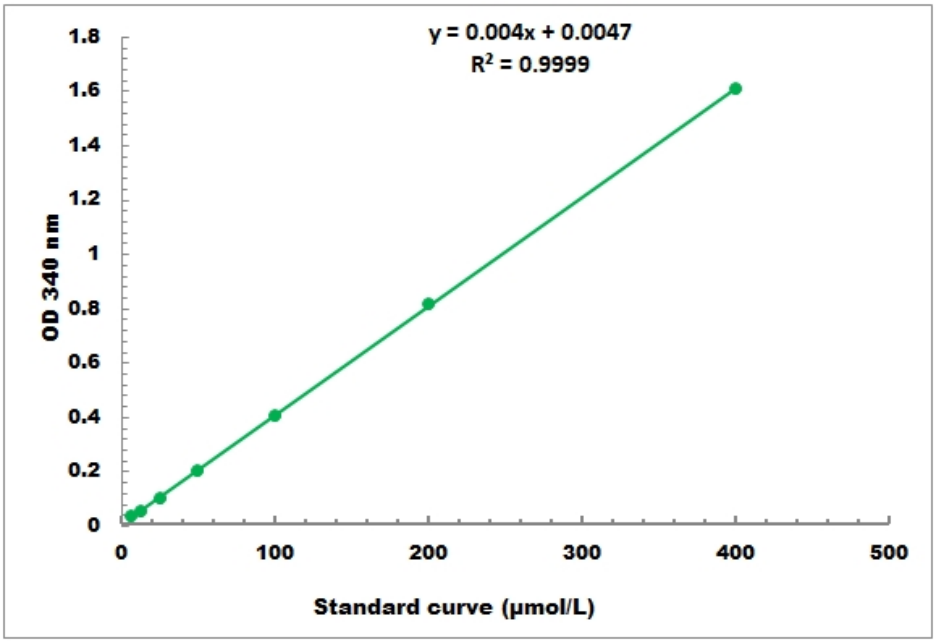
V_{Sample}: the volume of sample, 0.01 ml;

V_{Assay}: the volume of Assay buffer, 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: 4 μmol/L - 400 μmol/L