Technical Manual

Glutathione Reductase (GR) Activity Assay Kit

- Catalogue Code: MAES0088
- Size: 100 Assays
- Research Use Only

1. Key features and Sample Types

Detection method:

Colorimetric method

Specification:

100 Assays

Range:

6.2-320 U/L

Sensitivity:

6.2 U/L

Storage:

2-8°C and -20°C for 6 months

Expiry:

See Kit Label

Experiment Notes:

This kit is for **research use only.**

Instructions should be strictly followed. Changes of operation may result in unreliable results.

The validity of kit is 6 months.

Do not use components from different batches of kit.

2. Background

Glutathione reductase is a flavoprotein oxidoreductase, a homodimerase with a molecular weight between 100 kDa and 150 kDa, which is widely present in prokaryotes and eukaryotes. There are two isomers: GR2 is the isomer present in the cytoplasm, GR1 is the isomer present in the chloroplast and mitochondria.

3. Intended Use

This kit can be used to measure glutathione reductase (GR) activity in serum, plasma, tissue and cell samples.

4. Detection Principle

With the coenzyme as a hydrogen donor, GSSG can be reduced to GSH under the catalysis of GR. Then the GSH content is increased and NADPH decreased. The decrease of NADPH absorbance can be measured at 340 nm. The activity of GR can be calculated by detecting the change of NADPH.

5. Kit components & storage

Item	Specification	Storage
Buffer Solution	60 mL × 6 vials	2-8°C, 6 months
Substrate	Lyophilized × 8 vials	-20°C,6 months
Enzyme Reagent	Lyophilized × 4 vials	-20°C,6 months

Materials required but not supplied

- Micropipettor
- Incubator
- Centrifuge
- Spectrophotometer (340 nm)
- Tips (10 µL, 200 µL, 1000 µL)
- EP tubes (1.5 mL, 2 mL)
- Double distilled water
- Normal Saline (0.9% NaCl)
- PBS (0.01 M, pH 7.4)

6. Assay Notes:

1. Temperature has a great influence on the reaction system. Preheat the cuvette at 37°C when measuring the absorbance.

2. The detection procedure should be operated quickly. The operation steps should be operated carefully and contamination prevented. The time must be recorded accurately.

7. Reagent preparation:

1. Bring buffer solution to room temperature before use.

2. Preparation of substrate solution: Dissolve each vial of the substrate lyophilized with 1 mL of double distilled water before use. The prepared solution can be stored at 2-8°C for 2 days.

3. Preparation of enzyme solution: Dissolve each vial of the enzyme reagent lyophilized with 1 mL of double distilled water before use. The prepared solution can be stored at -20°C for 2 days.

4. Preparation of working solution: Mix buffer solution, substrate solution, enzyme solution according to the ratio of 2300: 60: 30, prepare the required amount before use. The prepared solution can be stored at 2-8°C for 4 days.

8. Sample Preparation

1. Serum sample:
Fresh blood should be incubated at 25°C for 30 min to clot the blood. Centrifuge the sample at 2000 g for 15 min at 4°C. Take the serum (which is the upper light yellow clarified liquid layer) and preserve it on ice before detection. If not detected on the same day, the serum can be stored at -80°C for a month.

2. Plasma sample:
Place the fresh blood sample into a tube of anticoagulant and centrifuge at 700-1000g for 10 min at 4°C. Take the plasma (which is the upper light yellow clarified liquid layer, don't take white blood cells and platelets in the middle layer) and preserve it on ice before detection. If not detected on the same day, the plasma can be stored at -80°C for a month.

3. Cell sample:
Collect the cells and wash the cells with PBS (0.01 M, pH 7.4) for 1~2 times. Centrifuge at 1000 g for 10 min and then discard the supernatant and keep the cell sediment. Add homogenization medium at a ratio of cell number (2×10^6): PBS (0.01 M, pH 7.4), contain 0.1 mM EDTA and 1.5% KCl (μL) =1: 200. Sonicate the sample on an ice water bath. Centrifuge at 10000 g for 10 min, then take the supernatant and preserve it on ice before detection. If not detected on the same day, the cells sample (without homogenization) can be stored at -80°C for a month.

4. Tissue sample:

Take 0.02-1g fresh tissue to wash with PBS (0.01 M, pH 7.4) at 2-8°C. Use filter paper to absorb excess water and weigh. Homogenize at the ratio of the volume of PBS (0.01 M, pH 7.4), contain 0.1 mM EDTA and 1.5% KCl (2-8°C) (mL): the weight of the tissue (g) =9:1, then centrifuge the tissue homogenate for 10 min at 10000 g at 4°C. Take the supernatant and preserve it on ice before detection. If not detected on the same day, the tissue sample (without homogenization) can be stored at -80°C for a month.

Sample Notes:

The concentration should be determined before preforming the assay. If the sample concentration is not within the range of the standard curve, users must determine the optimal sample dilutions for their particular experiments.

If the sample type is not included in the manual, a preliminary experiment is suggested to verify the validity.

Dilution of Samples:

Large variances in results may be seen when performing pre-experiments. Dilute the sample according to the result of the pre-experiment and the detection range (6.2-320 U/L).

The recommended dilution factor for different samples is as follows (for reference only).

Sample Type:	Dilution Factor
Human serum	1
Human plasma	1
Mouse serum	1
Mouse plasma	1
Rat serum	1
Rat plasma	1
10% Mouse liver tissue homogenization	1-3
10% Epipremnum aureum tissue homogenization	1

Note: The diluent is normal saline (0.9% NaCl) or PBS (0.01 M, pH 7.4);

9. Assay Protocol

Ambient Temperature: 25-30°C

Optimum detection wavelength: 340 nm

10. Operation Steps

1. Preheat the cuvette in incubator at 37°C for 5 min.

2. Set the spectrophotometer at 340 nm, prepare a couple of 1 cm optical path quartz cuvette, one is used for sample detection, another is used for setting to zero with double distilled water.

3. Add 65 μL of sample into the tube, then add 3120 μL of working solution, mix immediately and record the time at the same time.

4. Incubate at 37°C, measure the absorbance at 340 nm at 30 second (A_1) and 150 second (A_2), respectively. $\triangle A=A_1-A_2$

Operation Table

	Blank tube	Sample tube
Double distilled water (μL)	65	
Sample (μL)		65
Working solution (μL)	3120	3120
Mix immediately and record the time at the same time. Incubate at 37°C, measure the absorbance at 340 nm at 30 second (A_1) and 150 second (A_2), respectively. $\triangle A=A_1-A_2$.		

11. Calculations

1. Serum (plasma):
Definition: The amount of enzyme of 1 mmol of NADPH catalyzed by 1 L serum (plasma) per minute is defined as 1 unit.

$$\text{GR activity (U/L)} = \frac{\triangle A}{\varepsilon \times 1} \div t \times \frac{V_1}{V_2} \times f$$

2. Tissue and cell samples:
Definition: The amount of enzyme of 1 mmol of NADPH catalyzed by 1 g tissue protein per minute is defined as 1 unit.

$$\text{GR activity (U/gprot)} = \frac{\triangle A}{\varepsilon \times 1} \div t \times \frac{V_1}{V_2 \times C_{pr}} \times f$$

ΔA: $OD_{Sample} - OD_{Blank}$
ε: The extinction coefficient of 1 mM NADPH at 340 nm with 1 cm optical path quartz cuvette, 6.22 L/(mmol·cm)
1: Optical path, 1 cm
t: Reaction time, 2 min
V_1: The volume of sample in definition, 1 L=1000 mL
V_2: The volume of sample added to the reaction, 0.065 mL
f: Dilution factor of sample before test
C_{pr}: Concentration of protein in sample (gprot/L)

Email: info@assaygenie.com **Web:** www.assaygenie.com

12. Performance Characteristics

Detection Range	6.2-320 U/L
Sensitivity	6.2 U/L
Average recovery rate (%)	100
Average inter-assay CV (%)	2.5
Average intra-assay CV (%)	2.1

Analysis

Take 65 μL of human serum, carry the assay according to the operation table.

The results are as follows:

The A_1 of the blank is 0.470, the A_2 of the blank is 0.467, the A_1 of the sample is 0.483, the A_2 of the sample is 0.467, and the calculation result is:

$$\text{GR activity (U/L)} = \frac{(0.483-0.467)-(0.470-0.467)}{6.22\times1} \div 2 \times 1000 \div 0.065$$

$$= 16.08 \text{ (U/L)}$$

Safety Notes

Some of the reagents in the kit contain dangerous substances. Prevent touching skin and clothing.

Wash immediately with plenty of water if touching it carelessly.

All the samples and waste material should be treated according to the relevant rules of laboratory's biosafety.

Before the experiment, read the instructions carefully, and wear gloves and work clothes.

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