Glutathione Reductase (GR) Activity Assay Kit

Catalog No: E-BC-K099-S

Method: Colorimetric method

Specification: 100 Assays (Can detect 96 samples without duplication)

Instrument: Spectrophotometer

Sensitivity: 6.2 U/L

Detection range: 6.2-320 U/L

- Please kindly provide us the lot number (on the outside of the box) of the kit for more efficient service.

General information

▲Intended use

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

▲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.

▲Detection principle

With the coenzyme as hydrogen donor, GSSG can be reduced to GSH under the catalysis of GR. Then the GSH content 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.

$$GSSG + NADPH \xrightarrow{GR} 2GSH + NADP^{+}$$

▲Kit components & storage

Item	Component	Specification	Storage
Reagent 1	Buffer Solution	60 mL × 6 vials	2-8℃ , 6 months
Reagent 2	Substrate	Powder × 8 vials	-20℃ , 6 months
Reagent 3	Enzyme Reagent	Powder × 4 vials	-20℃ , 6 months
Note: The reagents must be stored strictly according to the preservation conditions in the above table. The reagents in different kits cannot be mixed with each other.			

▲Materials prepared by users

Instruments

Spectrophotometer (340 nm), Incubator, Vortex mixer, Micropipettor, Centrifuge

Consumptive material

Tips (10 μL, 200 μL, 1000 μL), EP tubes (1.5 mL, 2 mL, 5 mL)

Reagents

Double distilled water, Normal saline (0.9% NaCl), PBS (0.01 M, pH 7.4)

▲Safety data

Some of the reagents in the kit contain dangerous substances. It should be avoided to touch the 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.

▲Precautions

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

▲The key points of the assay

1. Just test one sample for each time.
2. Temperature has a great influence on the reaction system. Preheat the cuvette at 37°C when measuring the absorbance.
3. The detection procedure should be operated quickly. The operation steps should be operated carefully and avoid pollution and splash. The time must be recorded accurately.

Pre-assay preparation

▲Reagent preparation

1. Preparation of reagent 2

   Dissolve each vial of the powder with 1 mL of double distilled water fully before use. The prepared solution can be stored at 2-8℃ for 2 days.

2. Preparation of reagent 3

   Dissolve each vial of the powder with 1 mL of double distilled water fully before use. The prepared solution can be stored at -20℃ for 2 days.

3. Preparation of working solution

   Mix reagent 1, reagent 2, reagent 3 according to the ratio of 230: 6: 3 fully, prepare the needed amount before use. The prepared solution can be store at 2-8℃ for 4-5 days.

▲Sample preparation

The samples should be prepared as conventional methods. Also please refer to appendix II.

▲Dilution of sample

It is recommended to take 2~3 samples with expected large difference to do pre-experiment before formal experiment and 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 double distilled water or normal saline (0.9% NaCl).

Assay protocol	
Ambient temperature	25-30°C
Optimum detection wavelength	340 nm

Instructions for the use of transferpettor:

(1) Equilibrate the pipette tip in that reagent before pipetting each reagent.

(2) Don't add the liquid outside the tips into the reaction system when pipetting each reagent.

Assay protocol

▲Operating 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. $\Delta A=A_1-A_2$.

▲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. $\Delta A=A_1-A_2$.		

▲Calculation

1. Serum (plasma) sample:

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

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

2. Tissue and cells sample:

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

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

Note:

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)

▲Notes

1. This kit is for research use only.
2. Instructions should be followed strictly, changes of operation may result in unreliable results.
3. The validity of kit is 6 months.
4. Do not use components from different batches of kit.

Appendix I Performance characteristics

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

▲Example 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:

$$\text{GR activity (U/L)} = \frac{(0.483-0.467)-(0.470-0.467)}{6.22\times 1} \div 2 \times 1000 \div 0.065 = 16.08\ (\text{U/L})$$

Appendix II Sample preparation

The following sample pretreatment methods are for reference only.

▲Serum

Collect fresh blood and stand at 25°C for 30 min to clot the blood. Then centrifuge at 2000 g for 15 min at 4°C . Take the serum (which is the upper light yellow clarified liquid layer) to preserve it on ice for detection. If not detected on the same day, the serum can be stored at -80°C for a month.

▲Plasma

Take fresh blood into the tube which has anticoagulant, centrifuge at 700-1000 g 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) to preserve it on ice for detection. If not detected on the same day, the plasma can be stored at -80°C for a month.

▲Tissue sample

Take 0.02-1g fresh tissue to wash with PBS (0.01 M, pH 7.4) at 2-8°C . Absorb the water with filter paper and weigh. Homogenize at the ratio of the volume of homogenized medium (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 to preserve it on ice for detection. Meanwhile, determine the protein concentration of supernatant (E-BC-K318-M, E-BC-K168-S, E-BC-K165-S). If not detected on the same day, the tissue sample (without homogenization) can be stored at -80°C for a month.

▲Cells

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 (10^6): homogenization medium (μL) =1: 300-500. Sonicate or grind with hand-operated in ice water bath. Centrifuge at 10000 g for 10 min, then take the supernatant and preserve it on ice for detection. Meanwhile, determine the protein concentration of supernatant (E-BC-K318-M, E-BC-K168-S, E-BC-K165-S). If not detected on the same day, the cells sample (without homogenization) can be stored at -80℃ for a month.

Note:

1. Homogenized medium: PBS (0.01 M, pH 7.4), contain 0.1 mM EDTA and 1.5% KCl.

2. Homogenized method:

(1) Hand-operated: Weigh the tissue and mince to small pieces (1 mm^3), then put the tissues pieces to glass homogenized tube. Add homogenized medium into homogenized tube, place the tube into the ice bath with left hand, and insert the glass tamping rod vertically into the homogenized tube with the right hand to grind up and down for 6-8 min.

Or put the tissue into the mortar, and add liquid nitrogen to grind fully. Then add the homogenized medium to homogenize.

(2) Mechanical homogenate: Weigh the tissue to EP tube, add the homogenized medium to homogenize the tissue with homogenizer instrument (60 Hz, 90s) in the ice bath. (For samples of skin, muscle and plant tissue, the time of homogenization can be properly prolonged.)

(3) Ultrasonication: Treat the cells with ultrasonic cell disruptor (200 W, 2 s/time, interval for 3 s, the total time is 5 min).

▲Note for sample

1. Please predict the concentration before assaying. If the sample concentration is not within the range of the standard curve, users must determine the optimal sample dilutions for their particular experiments.
2. If the sample type is not included in the manual, a preliminary experiment is suggested to verify the validity.
3. If a lysis buffer is used to prepare tissue or cell homogenates, there is a possibility of causing a deviation due to the introduced chemical substance.