（FOR RESEARCH USE ONLY. DO NOT USE IT IN CLINICAL DIAGNOSIS !）

Catalog No：E-BC-K099-S

Specification：50 assays(48 samples)/100 assays(96 samples)

Measuring instrument：Spectrophotometer（340 nm）

Detection range：6.2-320 U/L

Elabscience®Glutathione Reductase (GR) Activity Assay Kit

This manual must be read attentively and completely before using this product.
If you have any problem, please contact our Technical Service Center for help：

Toll-free: 1-888-852-8623
Tell: 1-832-243-6086
Fax: 1-832-243-6017
Email: techsupport@elabscience.com
Website: www.elabscience.com

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

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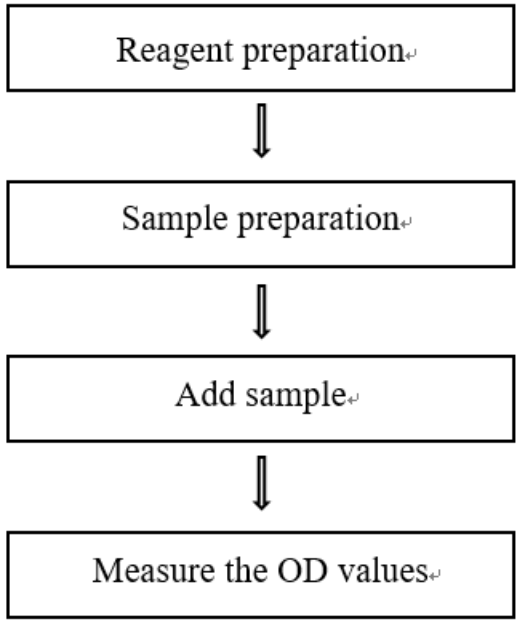
Assay summary

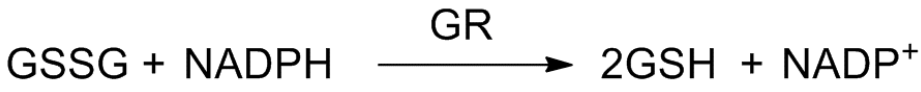


Intended use

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

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.



Kit components & storage

Item	Component	Size 1 (50 assays)	Size 2 (100 assays)	Storage
Reagent 1	Buffer Solution	60 mL × 3 vials	60 mL × 6 vials	2-8℃, 12 months
Reagent 2	Substrate	Powder × 4 vials	Powder × 8 vials	-20℃, 12 months
Reagent 3	Enzyme Reagent	Powder × 2 vials	Powder × 4 vials	-20℃, 12 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. For a small volume of reagents, please centrifuge before use, so as not to obtain sufficient amount of reagents.

Materials prepared by users

Instruments:

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

Reagents:

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

Reagent preparation

① Keep substrate and enzyme reagent on ice during use. Equilibrate buffer solution to room temperature before use.

② The preparation of substrate:
Dissolve one vial of substrate with 1 mL of double distilled water, mix well to dissolve. Store at 2-8℃ for 2 days.

③ The preparation of enzyme reagent:
Dissolve one vial of enzyme reagent with 1 mL of double distilled water, mix well to dissolve. Store at -20℃ for 2 days.

④ The preparation of working solution:
Before testing, please prepare sufficient working solution according to the test wells. For example, prepare 4780 μL of working solution (mix well 4600 μL of buffer solution, 120 μL of substrate and 60 μL of enzyme reagent, mix well. The working solution should be prepared on spot. Store at 2-8℃ for 4 days.

Sample preparation

① Sample preparation

Serum and plasma: detect directly. If not detected on the same day, the serum or plasma can be stored at -80℃ for a month.

Tissue sample:

① Harvest the amount of tissue needed for each assay (initial recommendation 20 mg).

② Wash tissue in cold PBS (0.01 M, pH 7.4).

③ Homogenize 20 mg tissue in 180 μL PBS (0.01 M, pH 7.4) with a dounce homogenizer at 4℃.

④ Centrifuge at 10000×g for 10 minutes at 4℃ to remove insoluble material. Collect supernatant and keep it on ice for detection.

⑤ Meanwhile, determine the protein concentration of supernatant (E-BC-K318-M).

Cell (adherent or suspension) samples:

① Harvest the number of cells needed for each assay (initial recommendation 1×10^6 cells).

② Wash cells with PBS (0.01 M, pH 7.4).

③ Homogenize 1×10^6 cells in 300 μL PBS (0.01 M, pH 7.4) with a ultrasonic cell disruptor at 4℃.

④ Centrifuge at 10000×g for 10 min at 4℃ to remove insoluble material. Collect supernatant and keep it on ice for detection.

⑤ Meanwhile, determine the protein concentration of supernatant (E-BC-K318-M).

② Dilution of sample

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). For the dilution of other sample types, please do pretest to confirm the dilution factor.

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℃ 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.

Operating steps

① Preheat the cuvette in incubator at 37℃ 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 ℃, measure the absorbance at 340 nm at 30 second (A_1) and 150 second (A_2), respectively. $\triangle A=A_1-A_2$.

Calculation

The sample:

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.

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

2. Tissue sample:

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

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

[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).

Appendix I Performance Characteristics

1. Parameter:

Intra-assay Precision

Three human serum samples were assayed in replicates of 20 to determine precision within an assay (CV = Coefficient of Variation).

Parameters	Sample 1	Sample 2	Sample 3
Mean (U/L)	15.50	148.00	243.50
%CV	2.3	1.8	2.2

Inter-assay Precision

Three human serum samples were assayed 20 times in duplicate by three operators to determine precision between assays.

Parameters	Sample 1	Sample 2	Sample 3
Mean (U/L)	15.50	148.00	243.50
%CV	2.4	2.6	2.5

Recovery

Take three samples of high concentration, middle concentration and low concentration to test the samples of each concentration for 6 times parallelly to get the average recovery rate of 100%.

	Sample 1	Sample 2	Sample 3
Expected Conc. （U/L）	85.4	168.5	262
Observed Conc. （U/L）	84.5	165.1	269.9
recovery rate(%)	99	98	103

Sensitivity

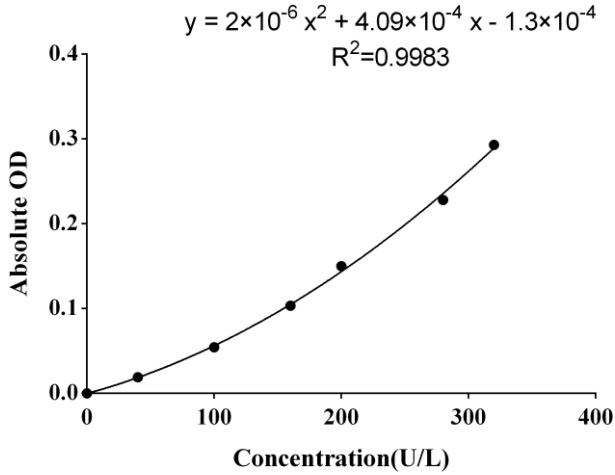
The analytical sensitivity of the assay is 6.2 U/L. This was determined by adding two standard deviations to the mean O.D. obtained when the zero standard was assayed 20 times, and calculating the corresponding concentration.

2. Standard curve:

(It doesn't need to prepare the standard curve for this kit and the provided standard curve is for reference only)

As the OD value of the standard curve may vary according to the conditions of the actual assay performance (e.g. operator, pipetting technique or temperature effects), so the standard curve and data are provided as below for reference only:

Concentration (U/L)	**0**	**40**	**100**	**160**	**200**	**280**	**320**	**8.0**
Average OD	0.137	0.286	0.410	0.634	0.759	0.840	0.948	1.058
Absoluted OD	0.000	0.149	0.273	0.497	0.622	0.703	0.811	0.921



Appendix II Example Analysis

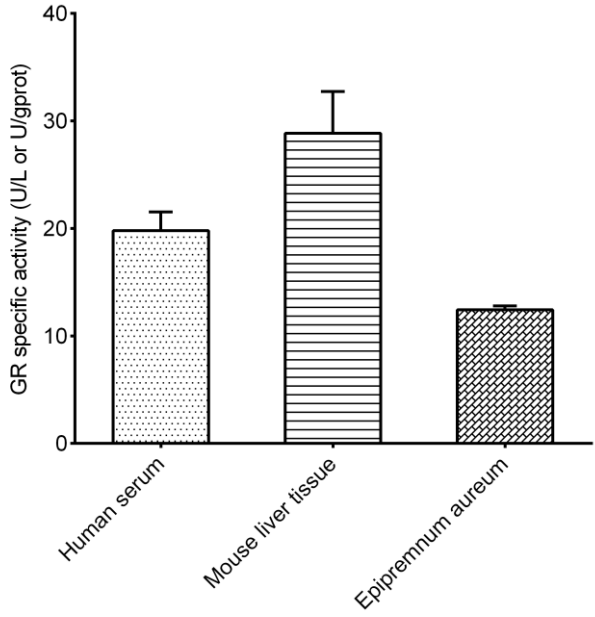
Example analysis:

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

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

Detect human serum, 10% mouse liver tissue homogenate (the concentration of protein in sample is 13.198 gprot/L, dilute for 2 times), 10% tissue homogenate of Epipremnum aureum leaves (the concentration of protein in sample is 1.924 gprot/L) according to the protocol, the result is as follows:



Statement

1. This assay kit is for Research Use Only. We will not response for any arising problems or legal responsibilities causing by using the kit for clinical diagnosis or other purpose.
2. Please read the instructions carefully and adjust the instruments before the experiments. Please follow the instructions strictly during the experiments.
3. Protection methods must be taken by wearing lab coat and latex gloves.
4. If the concentration of substance is not within the detection range exactly, an extra dilution or concentration should be taken for the sample.
5. It is recommended to take a pre-test if your sample is not listed in the instruction book.
6. The experimental results are closely related to the situation of reagents, operations, environment and so on. Elabscience will guarantee the quality of the kits only, and NOT be responsible for the sample consumption caused by using the assay kits. It is better to calculate the possible usage of sample and reserve sufficient samples before use.