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

Catalog No：E-BC-K099-M

Specification：48T(46 samples)/ 96T(94 samples)

Measuring instrument：Microplate reader (340 nm)

Detection range：1.87-150 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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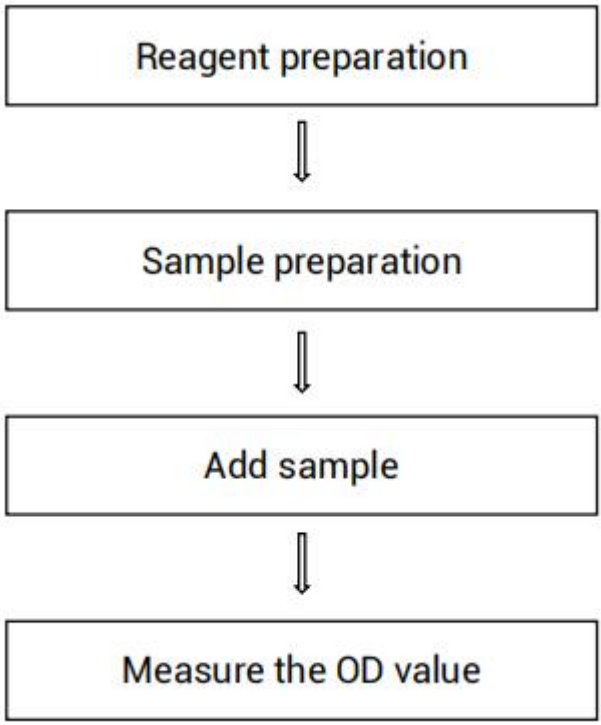
Assay summary

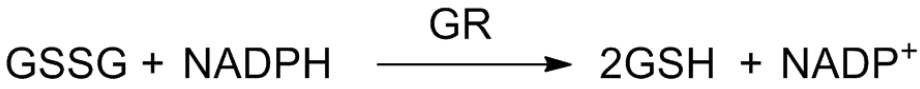


Intended use

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

Detection principle

With the reduced coenzyme (NADPH) as hydrogen donor, oxidized glutathione (GSSG) can be reduced to reduced glutathione (GSH). 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 (48T)	Size 2 (96T)	Storage
Reagent 1	Buffer Solution	14 mL × 1 vial	28 mL × 1 vial	2-8℃, 12 months
Reagent 2	Substrate	Powder × 1 vial	Powder × 2 vials	-20℃, 12 months
Reagent 3	Enzyme Reagent	Powder × 1 vial	Powder × 2 vials	-20℃, 12 months
	UV Microplate	96 wells		No requirement
	Plate Sealer	2 pieces		
	Sample Layout Sheet	1 piece		

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:

Microplate reader (340 nm), Incubator, Vortex mixer, Micropipettor, Centrifuge, Magnetic stirrer

Reagents:

Double distilled water, Normal saline (0.9% NaCl)，Deionized water

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 working solution：
Dissolve one vial of substrate with 1 mL of double distilled water, mix well to dissolve. Store at 2-8°C for 4 days.

③ The preparation of enzyme working solution：
Dissolve one vial of enzyme reagent with 1 mL of double distilled water, mix well to dissolve. Store at -20°C for 7 days.

④ The preparation of working solution：
Before testing, please prepare sufficient working solution according to the test wells. For example, prepare 245 μL of working solution (mix well 230 μL of buffer solution, 6 μL of substrate working solution and 9 μL of enzyme working solution). The working solution should be prepared on spot. Store at 2-8°C 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 normal saline (0.9% NaCl) with a dounce homogenizer 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).

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 200 μL normal saline (0.9% NaCl) 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	2-8

Note: The diluent is 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 Strictly control reaction time and operation time.

② If the A_2 value of the sample is less than 0.2, the sample should be diluted.

Operating steps

① Blank wells: Add 10 μL of double distilled water to the corresponding wells.

Sample wells: Add 10 μL of sample to the corresponding wells.

② Add 200 μL of working solution to each well.

③ Measure the OD value (A_1) of each well at 340 nm.

④ Incubate at 37°C for 15 min and measure the OD value (A_2) of each well at 340 nm.

Calculation

The sample:

1. Serum (plasma) sample:

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

$$\text{GR activity (U/L)} = \frac{\Delta A}{\varepsilon \times 0.6} \div t \times \frac{V_1}{V_2} \times 1000 \times f$$

2. Tissue and cell sample:

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

$$\text{GR activity (U/gprot)} = \frac{\Delta A}{\varepsilon \times 0.6} \div t \div C_{pr} \times \frac{V_1}{V_2} \times 1000 \times f$$

[Note]

ΔA: $\Delta A_{Sample} - \Delta A_{Blank}$, $\Delta A_{Sample} = A_1 - A_2$, $\Delta A_{Blank} = A_1 - A_2$.

ε: The extinction coefficient of 1 mM NADPH at 340 nm with 0.6 cm optical path quartz cuvette, 6.22 L/(mmol•cm).

1: Optical path, 0.6 cm.

t: Reaction time, 15 min.

V_1: The volume of reaction system, 210 μL.

V_2: The volume of sample added to the reaction system, 10 μL.

f: Dilution factor of sample before test.

C_{pr}: Concentration of protein in sample (gprot/L).

1000：1 mmol = 1000 μmol

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)	30.00	80.00	120.00
%CV	3.9	2.5	1.5

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)	30.00	80.00	120.00
%CV	2.1	5.6	6.8

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 101%.

	Sample 1	Sample 2	Sample 3
Expected Conc. (U/L)	30	80	120
Observed Conc. (U/L)	28.8	81.6	126
Recovery rate (%)	96	102	105

Sensitivity

The analytical sensitivity of the assay is 1.87 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.

Appendix Π Example Analysis

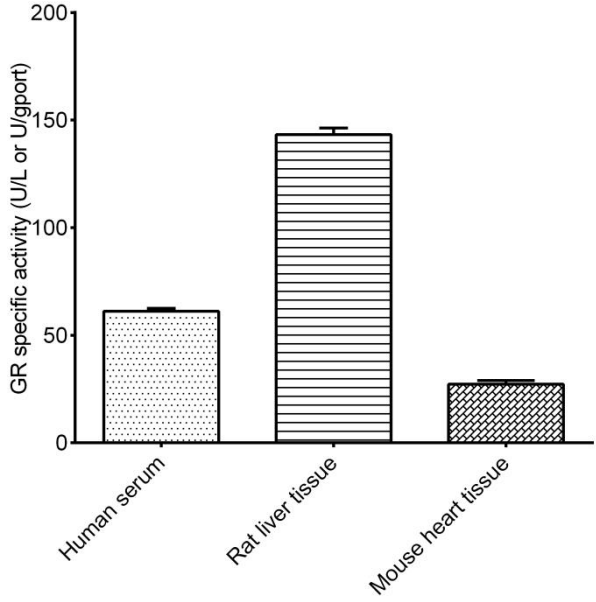
Example analysis：

Take 10 μL of human serum, carry the assay according to the operation steps. The results are as follows:

The A_1 of the blank is 0.845, the A_2 of the blank is 0.832, the A_1 of the sample is 0.833, the A_2 of the sample is 0.659, and the calculation result is:

$$\text{GR activity (U/L)} = \frac{(0.833 - 0.659) - (0.845 - 0.832)}{6.22 \times 0.6} \div 15 \times 1000 \times 21 = 60.39\ \text{U/L}$$

Detect human serum, 10% rat liver tissue homogenate (the concentration of protein in sample is 13.20 gprot/L, dilute for 4 times) and 10% mouse heart tissue homogenate (the concentration of protein in sample is 6.90 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.