



Glutathione Reductase (GR) Assay Kit

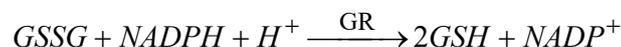
(Cat.No:BC098 Size: 50T/48S)

1. Significance of Determination

Glutathione reductase is a flavoprotein enzyme, and each molecule of the enzyme protein contains one molecule of FAD. Hydrogen is supplied by coenzyme NADPH, which catalyzes the oxidation of glutathione (GSSG) to reduce it to reduced glutathione (GSH). Reduced glutathione (GSH) can make enzymes with sulfhydryl groups (-SH) remain in a reduced state and active state, maintain the integrity of red blood cell membranes, and prevent the oxidation of hemoglobin. GR is located in the mitochondrial and cytosolic parts. Since the tissue cells of various organs generally contain GR, GR plays a crucial role in the oxidation-reduction reactions of the body.

2. Principle (Ultraviolet colorimetry)

Under the catalysis of glutathione reductase (GR), oxidized glutathione (GSSG) is reduced to reduced glutathione (GSH) by NADPH providing hydrogen. As a result, GSH increases while NADPH decreases. The absorbance value of NADPH at 340nm can be detected to show a decrease.



By detecting the changes in NADPH, the activity of GR can be calculated.

3. Reagent Composition (The kit is valid for 6 months)

Reagent 1: 60mL×2 bottles, Store at 4°C;

Reagent 2: Powder×4 vials, Store at 4°C; for each use, add 1 mL of double-distilled water and dissolve thoroughly before storage at 4°C.

Reagent 3: Powder×2 vials, Store at 4°C; before use, add 1 mL of double-distilled water to each vial and dissolve thoroughly before use; store at -20°C or below.

Preparation of the working solution: Prepare it in the ratio of reagent 1:reagent 2: reagent 3 = 2300 : 60 : 30. Use as much as needed, prepare immediately as you go. Store the remaining portion at 4°C for 4 to 5 days (preheat at 37°C for 5 minutes before use).

4. Determination of GR activity

1. Sample pre-treatment:

Serum (plasma) and other liquid samples: Use directly.

Cell culture medium: Centrifuge at 4000 rpm for 5 minutes, then take the supernatant for



testing.

Animal tissue samples: Accurately weigh the tissue. Add 9 times the volume of normal saline at a ratio of weight (g): volume (mL) = 1:9. Perform mechanical homogenization under an ice-water bath. Centrifuge at 4000 revolutions per minute for 10 minutes. Take the supernatant (the supernatant needs to be measured for its protein concentration. The protein determination kit is available from our company). This will be the sample to be tested.

Plant tissue samples: Method 1: First, rinse plant tissues thoroughly with PBS, then blot dry with absorbent paper. Chop the tissues into small pieces and transfer to a mortar, grind into a fine powder using liquid nitrogen. Weigh the plant powder, then add PBS at a weight-to-volume (w/v) ratio of 1:9 (i.e., 9 mL of PBS per gram of powder). Vortex vigorously (or grind with a homogenizer) for 1 minute, followed by centrifugation at 4000 rpm for 10 minutes. Collect the supernatant for subsequent assay. **Method 2:** After washing the plant tissues and blotting dry, weigh them directly. Add PBS at a weight-to-volume (w/v) ratio of 1:9 (i.e., 9 mL of PBS per gram of tissue), then perform mechanical homogenization on an ice-water bath. Centrifuge the homogenate at 4000 rpm for 10 minutes, and collect the supernatant for subsequent assay. **(Note: Plants with a higher moisture content are typically processed using Method 2, while those with low moisture content or dry samples are recommended to be processed using Method 1.)**

Cell sample: After collecting the cells, each sample of cells (the cell count should not be less than 10^6 , the more the better) is added with 0.3 mL of physiological saline (or PBS). Under an ice water bath, ultrasonic disruption is performed (power 200-300W, run for 5 seconds, with a 15-second interval, repeated 3-5 times). Centrifuge at 4000 rpm for 10 minutes, and take the supernatant (the supernatant needs to be measured for its protein concentration. Protein determination reagents are available from our company). It is ready for testing.

2. Operation Process:

- ①. Set the ultraviolet spectrophotometer at 340 nm, with a 1 cm quartz cuvette, and zero the instrument using double-distilled water; (prepare two quartz cuvettes, one for zeroing and the other for measurement.)
- ②. Add 50 μ L of the sample to the corresponding numbered test tube, then draw 2.4 mL of the working solution and pour it into the tube. Mix quickly and start timing.
- ③. Immediately pour it into the quartz cuvette, and measure with the UV spectrophotometer at 340 nm. Read the absorbance value A1 at 30 seconds.
- ④. Pour this reaction solution into the original test tube and place it in a water bath at



37°C. Then, quickly pour it into the quartz cuvette at 2 minutes and 20 seconds, and read the absorbance value A2 at 2 minutes and 30 seconds.

- ⑤. Calculate the difference in absorbance values for two measurements ($\Delta A = A1 - A2$).

[Note]: The timing for the first reading can be flexible (20 seconds, 40 seconds, etc., but not too long). As long as the time difference between A1 and A2 is 2 minutes, it is sufficient. If the values of A1 and A2 are close, you can increase the sample volume or extend the reaction time (such as 5 minutes or 10 minutes).

5. Definition and Calculation of GR Vitality

- ①. Definition of GR enzyme activity unit for liquid samples such as serum (or plasma) (including cell culture supernatant): The amount of enzyme required to cause a 1 mM change in the concentration of the substrate NADPH in the reaction system per liter of liquid sample per minute is defined as one enzyme activity unit (U).

Calculation formula:

$$\text{GR activity in blood serum(or plasma)(U/L)} = \frac{\Delta A}{6.22 \times d} \div T \div V_{\text{sample}} \times N$$

Definition of GR enzyme activity unit for tissue and cell samples: One enzyme activity unit (U) is defined as the amount of enzyme (per gram of protein in the sample) required to induce a 1 mM change in the concentration of the substrate NADPH in the reaction system per minute.

Calculation formula:

$$\text{GR activity in blood serum(or plasma)(U/L)} = \frac{\Delta A}{6.22 \times d} \div T \div (V_{\text{sample}} \times C_{\text{pr}})$$

In the formula above:

6.22: Extinction coefficient of NADPH at 340 nm with a 1 cm light path.

d: Cuvette light path, 1 cm.

T: Reaction time, 2 min.

V_{sample} : Sample volume, 0.05×10^{-3} L.

N: Dilution factor of the sample before testing.

Cpr: Protein concentration of the tissue homogenate, gprot/mL (where "prot" denotes protein).

6. For example

Example 1: Add 50 μ L of rat plasma to a test tube, then add 2.4 mL of the Working Solution. Mix thoroughly, then transfer the mixture into a 1 cm light path quartz cuvette. Measure the absorbance at 340 nm, and record the absorbance value (A1) as 0.441 at 30 seconds. Incubate the mixture at 37°C for exactly 2 minutes. Quickly retrieve the cuvette, wipe it dry, and measure the absorbance again at 340 nm. Record the absorbance value (A2) as 0.431 at 2 minutes and 30 seconds.

The calculation is as follows:



$$\text{Rat plasma GR activity (U/L)} = \frac{0.441 - 0.431}{6.22 \times 1} \div 2 \div (0.05 \times 10^{-2}) = 16.08 \text{ U/L}$$

Example 2: Add 50 μL of 4% mouse liver tissue homogenate to a test tube, then add 2.4 mL of the Working Solution. Mix thoroughly and transfer the mixture into a pre-warmed quartz cuvette with a 1 cm light path. Measure the absorbance at 340 nm and record the absorbance value (A1) as 0.504 at 30 seconds.

Incubate the cuvette in a 37°C water bath for exactly 2 minutes. Quickly retrieve the cuvette and measure the absorbance again at 340 nm, recording the absorbance value (A2) as 0.491 at 2 minutes and 30 seconds. Meanwhile, the protein concentration of the 4% liver tissue homogenate was determined to be 2.96×10^{-3} gprot/mL.

The calculation is as follows:

$$\text{GR activity in tissue sample (U/gprot)} = \frac{0.504 - 0.491}{6.22 \times 1} \div 2 \div (0.05 \times 2.96 \times 10^{-3}) = 7.06 \text{ U/gprot}$$