Glutathione Reductase Activity Coefficient Test Kit

(Cat/No.: BC044 Pack: 100T/48S GRAC)

1. Composition and preparation

Reagent 1: Buffer, 32mL × 1 bottle, stored at 4°C.

Reagent 2: Substrate stock solution, 0.22mL × 1 bottle, stored at -20°C in the dark.

Substrate diluent, 2.2 mL × 1 bottle, stored at -20°C.

Preparation of substrate application solution: Before use, dilute 10 times the substrate stock solution: substrate diluent = 1:9 to prepare a substrate application solution. Store the unused application solution at 4°C within three days. Store at -20℃ for more than 1 day.

Reagent 3: matrix powder × 2 bottles, stored at -20°C in the dark.

Matrix diluent, 2.5mL × 2 bottles, stored at -20°C.

Preparation of matrix application solution: Before use, add 1 tube of matrix powder to 1 bottle of matrix diluent and fully dissolve it to prepare the matrix application solution. Store the unused application solution at 4°C within three days and -20°C for more than three days. .

Reagent 4: Accelerator powder × 2 bottles, stored at -20°C away from light.

Accelerator diluent, 1.3mL × 2 bottles, stored at -20°C.

Preparation of accelerator application liquid: Before use, add 1 bottle of accelerator powder to 1 bottle of accelerator diluent and fully dissolve it to prepare an accelerator application liquid. Store the unused application liquid at 4°C within three days. For more than three days - Store at 20℃.

Reagent 5: Precipitating agent, 40mL × 2 bottles, stored at 4°C.

Reagent 6: 2 bottles of chromogen powder, stored at 4°C in the dark.

Chromogen diluent, 50mL x 2 bottles, stored at 4°C in the dark.

Preparation of developer application solution: Before use, add 1 tube of developer powder to 1 bottle of developer diluent and fully dissolve it to prepare a developer application solution. Store in the dark at 4°C.

2. Steps

Phosphotungstic acid can be reduced by Cys and form tungsten blue, which has an absorption peak at 600 nm. Cys content can be calculated with the absorbance at 600 nm.

	Blank tube	Sample tube	Sample blank tube
Double distilled water （μL）	270		
Sample to be tested （μL）		300	300
Reagent 1 （μL）	300	250	300
Reagent 2 （μL）		20	20
Reagent 3 （μL）	50	50	50
Reagent 4 （μL）	50	50	
Incubate at 37 ° C for 30 minutes			
Reagent 5 （μL）	800	800	800
Mix well and centrifuge at 4000 rpm for 10 minutes.			
Supernatant （μL）	300	300	300
Reagent 6 （μL）	1000	1000	1000

Mix well, let stand for 5 minutes, use 420nm wavelength, 0.5cm optical diameter cuvette, double-distilled water to zero, and measure the absorbance OD value of each tube.

[Note]: * Blank tubes are very stable, only 1 to 2 need to be made.

**See the appendix for sample pre-processing.

3. Calculation formula

Glutathione reductase activity coefficient $= \frac{A_{sample} - A_{blank}}{A_{sample\ blank} - A_{blank}}$

4. Determination principle

Glutathione reductase (GR) can catalyze the reduction of oxidized glutathione (GSSG) to reduced glutathione (GSH) in the presence of reduced coenzyme II. The prosthetic group of this enzyme is yellow. adenine dinucleotide (FAD). Therefore, when riboflavin deficiency leads to FAD deficiency, the activity of this enzyme can be reduced. The nutritional status of riboflavin in the body is evaluated by measuring the glutathione reductase activity coefficient.

5. Detection meaning

In the body, riboflavin combines with enzyme proteins in the form of FAD to form various flavoproteins, which participate in the redox process as an electron transfer system. When riboflavin is deficient, a series of symptoms occur on the skin, mucous membranes, and eyes. In order to detect riboflavin deficiency early and take timely prevention and control measures, it is necessary to assess riboflavin nutritional status.

When riboflavin is deficient, glutathione activity coefficient increases rapidly, but after riboflavin supplementation, it drops to normal. Therefore, the Glutathione Reductase Activation Coefficient (GRAC) is an accurate indicator for assessing the total level of riboflavin in the body when chronic riboflavin deficiency occurs. The use of glutathione reductase activity coefficient (GRAC) value to evaluate riboflavin nutritional status has the advantages of being sensitive, stable, accurate, trace, and able to reflect the metabolic utilization in the body.

Appendix I: Determination of glutathione reductase activity coefficient in whole blood

I. Sample pre-processing:

Take 10 μL of fresh whole blood sample, add 990 μL of double-distilled water, vortex and mix for 1 minute to cause hemolysis, prepare a 100-fold diluted hemolysate, and add the sample according to Ian's operation table. (Look at the light to see if it is translucent. If it is not translucent or turbid, centrifuge at 4000 rpm for 5 minutes and take the supernatant for measurement)

II. Calculation examples:

Take 10 μl of the chicken whole blood sample and add 990 μl of the extraction solution, vortex and mix for 1 minute to cause hemolysis, prepare a 100-fold diluted hemolysate, and then take a 100-fold diluted hemolysate for measurement. The measured absorbance of the blank tube is 0.038, and the absorbance of the measuring tube is

0.038. 0.085, and the absorbance of the blank tube is measured to be 0.042, then the

calculation result is: Glutathione reductase activity coefficient

$$= \frac{0.085 - 0.038}{0.042 - 0.038} = 12.5$$

Appendix II: Determination of glutathione reductase activity coefficient in serum

I. Sample pre-processing:

Take 200 μL of serum sample, add 600 μL of physiological saline, dilute it 1:3, and then add the sample according to the operation table.

II. Calculation examples:

Take 200 μl of chicken serum sample and add 600 μl of physiological saline, dilute it 1:3 and then measure. The measured absorbance of the blank tube is 0.038, the measured absorbance of the measuring tube is 0.088, and the measured absorbance of the blank tube is 0.042. The calculation result is:

Appendix III: Determination of glutathione reductase activity coefficient in tissues

I. Sample pre-processing:

Accurately weigh the tissue, according to the ratio of weight (g): volume (mL) = 1:9, add 9 times the volume of normal saline, prepare 10% tissue homogenate under ice water conditions, centrifuge at 3500 rpm After 10 minutes, take the supernatant and dilute it 5 times with physiological saline to prepare 2% tissue supernatant. Add samples according to the operation table.

II. Calculation examples:

Take a rat liver tissue sample, prepare a 10% homogenate with normal saline, centrifuge to get the supernatant, and then use normal saline at a ratio of 1:4, that is, 5 times diluted to a 2% homogenate for measurement. The measured The absorbance of the blank tube is 0.038, the absorbance of the measuring tube is 0.141, and the absorbance of the blank measuring tube is 0.062, the calculation result is:

Glutathione reductase activity coefficient $= \frac{0.141 - 0.038}{0.062 - 0.038} = 4.29$