Glutathione Reductase Activation Coefficient Assay Kit

Detection Equipment: Spectrophotometer/Microplate Reader

Catalog Number: AK0901-100T-48S

Size: 100T/48S

Components:Please carefully check the volume of the reagent and the volume in the bottle before use. If you have any questions, please contact Solarbio staff in time.

Reagent name	Size	Preservation condition
Reagent I	Liquid 65 mL×1	2-8℃ storage
Reagent II	Powder ×1	2-8℃ storage
Reagent III A	Powder ×1	-20℃ storage
Reagent III B	Liquid 1 mL×1	2-8℃ storage
Reagent IV	Liquid 1.2 mL×1	2-8℃ storage
Reagent V	Powder ×1	-20℃ storage
Reagent VI	Liquid 20 mL×1	2-8℃ storage
Reagent VII	Liquid 25 mL×1	2-8℃ storage

Solution reparation :

1. Reagent II: Add 1.2mL distilled water before use, dissolve fully, and storage for 4 weeks at 2-8℃;
2. Reagent III: Before use, take Reagent III A and add 0.537mL Reagent III B, dissolve fully, can be storage for 4 weeks at -20℃, to avoid repeated freezing and thawing;
3. Working liquid: Prepare working liquid according to the sample size of Reagent I: Reagent II: Reagent III: Reagent IV = 40μL: 10μL: 4μL: 10μL (64μL, 1T) before clinical use.
4. Reagent V: Add 1.2mL distilled water before use, dissolve fully, can be storage separately at -20℃ for 4 weeks to avoid repeated freezing and thawing;
5. Reagent V working liquid: Prepared according to the sample size of Reagent V: distilled water =5μL: 95μL (0.1mL, 10T) before clinical use.

Description:

Glutathione Reductase (GR) is a flavin protein oxidoreductase widely present in eukaryotes and prokaryotes. This enzyme is cobased on riboflavin adenine dinucleotide (FAD), a riboflavin derivative. Catalyzed NADPH reduction oxidized glutathione (GSSG) to produce reduced glutathione (GSH), maintaining sulfhydryl group and membrane protein in reduced state. When riboflavin was lacking in vivo, FAD content decreased correspondingly, GR activity also decreased, glutathione reductase activity Coefficient (GRAC) increased rapidly, and clinical symptoms such as cheilitis, keratitis, conjunctivitis appeared. Therefore, the use of GRAC in the evaluation of riboflavin nutritional status is of great significance for early detection and prevention of patients with deficiency.

GR catalyzes NADPH reduction of GSSG to produce GSH, which reacts with 5,5 '-dithiobis-2-nitrobenoic acid (5,5'-dithiobis-2-nitrobenoic acid, DTNB) to produce 2-nitro-5-mercaptobenic acid. 2-nitro-5-mercaptobenzoic acid has a characteristic absorption peak at 412nm, and the activity of GR can be calculated by the change of absorbance at 412nm. According to the change of GR activity before and after adding FAD, GRAC can be calculated.

$$GSSG + NADPH \xrightarrow{\text{Glutathione Reductase}} GSH + NADP^+$$

$$GSH + DTNB \xrightarrow{\text{FAD}} TNB\ (412nm)$$

Note: Before the experiment, it is recommended to select 2-3 sample with large expected differences for pre-experiment. If the absorption value of the sample is not within the measurement range, it is recommended to dilute or increase the sample size for detection.

Reagents and Equipment Required but Not Provided:

Spectrophotometer/microplate reader, low temperature centrifuge, analytical balance, water bath/constant temperature incubator, micro glass cuvette/96 well flat-bottom plate, adjustable pipette, mortar/homogenizer/cell ultrasonic crusher, ice and distilled water.

Protocol:

I. Sample processing (The sample size to be tested can be appropriately adjusted, and the specific proportion can be referred to the literature)

1. **Tissue sample:** Add Reagent I according to mass (g) : Reagent I volume (mL) = 1:5 ~10 (it is recommended to weigh 0.1g sample and add 1.0mL Reagent I), after ice bath homogenization, centrifuge at 4℃, 12000rpm for 10min, discard precipitation, take supernatant and put it on ice to be measured.
2. **Cell sample:** According to the number of cells (10^4) : reagent volume (mL) =500~1000: 1 add Reagent I (it is recommended to add 1.0mL Reagent I to 5 million cells), break the cells in an ice bath ultrasonic (power 200W, ultrasonic 3s, interval 7s, total time 3min), then centrifuge at 4℃, 12000rpm for 10min, discard the precipitation, take the superserum and put it on ice to be measured.
3. **Whole blood/red blood cell:** It is recommended to take 10μL whole blood/red blood cells, add 990μL Reagent I, fully hemolute and mix, centrifuge at 4℃, 4000rpm for 10min, discard precipitation, take supernatant and put it on ice to be measured.
4. **Serum/plasma and other liquid sample:** Direct measurement. If there is turbidity, centrifuge, take the supernatant and put it on the ice to be measured.

II. Measurement Steps

1. Spectrophotometer/microplate reader for more than 30min, adjust the wavelength to 412nm, and zero the distilled water.
2. Preheat the working liquid at 37℃ for 10min.
3. Operating table: (Add the following reagents to 1.5mL EP tube)

Reagent name（μL）	Test tube	Control tube	Blank tube
Sample	60	60	-
Distilled water	-	10	60
Working solution	64	64	64

Reagent V working solution	10	-	10
The mixture was mixed and incubated at 37 ° C for 30min			
Reagent VI	160	160	160
The mixture was mixed, centrifuged at 4000rpm for 10min, and the supernatant was taken in an EP tube to be measured.			
Supernatant fluid	60	60	60
Reagent VII	200	200	200
The mixture was mixed and allowed to stand for 5min at room temperature, and 200μL of the reaction solution was placed in micro glass cuvette/96 well flat-bottom plate to determine the absorbance value at 412, which was recorded as A_{text}, $A_{control}$, and A_{blank}, respectively. One control tube should be set for each assay tube, and blank tubes only need to be measured once or twice.			

III. Calculations

$$GRAC = (A_{text} - A_{blank}) \div (A_{control} - A_{blank})$$

Note:

1. It is recommended to prepare samples with normal riboflavin levels for testing, so as to facilitate comparison with riboflavin deficient samples; If there is no sample with normal riboflavin level, the GRAC of the sample with normal riboflavin level can be 0.9-1.2.
2. If the absorption value of the sample measurement tube is greater than 1.5, it is recommended to dilute the sample with reagent 1 for determination; If the absorbance value of the sample measuring tube is close to that of the blank tube, the sample quality can be appropriately increased for re-extraction or the sample volume in the sample adding table, and the distilled water in the tube and the blank tube should be adjusted accordingly.
3. The protein concentration of the sample should be determined by itself. Because the extract contains a certain concentration of protein (about 0.11mg/mL), it is necessary to subtract the protein content of the extract itself when determining the protein concentration of the sample.

Experimental example:

1. Take 0.1051g rat kidney sample, add 1mL reagent to the ice bath homogenate, centrifuge and take supernatant, according to the measurement procedure, use 96 well flat-bottom plate to measure: A_{text} =0.370, $A_{control}$ =0.360, A_{blank} =0.107, the calculation is as follows:
   $GRAC = (A_{text} - A_{blank}) \div (A_{control} - A_{blank}) = 1.040$.
2. Take 10μL human red blood cells, add 990μL reagent 1, fully hemolysis and mix well, centrifuge and take supernant, according to the measurement procedure, use 96 well flat-bottom plate to measure: A_{text} =0.479, $A_{control}$ =0.461, A_{blank} =0.107, the calculation is as follows:
   $GRAC = (A_{text} - A_{blank}) \div (A_{control} - A_{blank}) = 1.051$.
3. Take equine serum sample, according to the measurement procedure directly, use 96 well flat-bottom plate to measure: A_{text} =0.397, $A_{control}$ =0.392, A_{blank} =0.107, and the calculation was as follows:
   $GRAC = (A_{text} - A_{blank}) \div (A_{control} - A_{blank}) = 1.018$.

References：

[1] Sauberlich H E, Judd J H, Nichoalds G E, et al. Application of the erythrocyte glutathione reductase assay in evaluating riboflavin nutritional status in a high school student population [J]. The American Journal of Clinical Nutrition, 1972, 25(8): 756-762.

[2] Gu CF, Chen YZ, Wang ZY. A study of the activation coefficients of blood glutathione reductase in the evaluation of experimental riboflavin deficiency [J]. Acta Nutrimenta Sinica, 1981, 3(4): 251-260.

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[4] He J, Hu ML, H YM, et al. Study on the optimum conditions for determination of glutathione reductase activity coefficient in whole blood [J]. Chinese Journal of Public Health, 2002, 18(5): 615-616.