Reduced Glutathione (GSH) Content Assay Kit

Note: Take two or three different samples for prediction before test.

Operation Equipment: Spectrophotometer

Catalog Number: BC1170

Size: 50T/48S

Product Composition: Before use, please carefully check whether the volume of the reagent is consistent with the volume in the bottle. If you have any questions, please contact Solarbio staff in time.

Reagent name	Size	Preservation Condition
Reagent I	Liquid 60 mL×1	2-8℃
Reagent II	Liquid 50 mL×1	2-8℃
Reagent III	Liquid 15 mL×1	2-8℃
Standard	Powder×1	2-8℃

Solution Preparation:

1. Standard: 10mg of reduced glutathione (GSH). Add 1 mL distilled water to dissolve before use. The reagent can be stored at 2-8℃ for 6 weeks.

Product Description

Glutathione is a natural tripeptide composed of glutamic acid (Glu), cysteine (Cys) and glycine (Gly). It is a kind of compound containing sulfhydryl group (-SH), which widely exists in animal tissue, plant tissue, microorganism and yeast. Glutathione can react with 5,5'-dithiobis-(2-nitrobenzoic acid) (DTNB) to produce 2-nitro-5-mercaptobenzoic acid and glutathione disulfide (GSSG). 2-nitro-5-mercaptobenzoic acid is a yellow product with the maximum absorption at 412 nm.

Technical Specifications

Minimum Detection Limit：2.67 μg/mL；Linear Range：3.125-250 μg/mL

Reagents and Equipment Required but Not Provided

Analytical balance, mortar/homogenizer, low temperature centrifuge, water bath, adjustable pipette, spectrophotometer, 1 mL glass cuvette, ice and distilled water.

Procedure

I. Sample preparation

1. Tissue sample

According to the ratio of tissue mass (g) : reagent volume (mL) of 1 : 5 ~ 10 (it is recommended to weigh about 0.1g of tissue and add 1mL of reagent I) for ice bath homogenization (homogenizer / mortar pre-cooled on ice in advance). 12000 g, centrifuged at 4 °C for 10 min, and the supernatant is placed at 4 °C for test. If the test cannot be completed temporarily, it can be stored at-80 °C (can be stored for 3 days).

2. Blood sample

(1) Plasma: The collected anticoagulant blood is centrifuged at 4 °C, 600g for 10 minutes, and the upper plasma was taken into another test tube, and an equal volume of reagent I is added, and be boiled for 5min (wrapped and sealed to prevent explosion). After centrifugation at 12000 g for 10 minutes at 4℃, the supernatant is transferred into a new test tube and placed at 4 °C for testing. If the test cannot be completed temporarily, it can be stored at-80 °C for 3 days.

(2) Blood cells: The collected anticoagulant blood is centrifuged at 4 °C, 600g for 10 minutes, the upper plasma is discarded and washed three times with 3 times the volume of PBS (re-suspended blood cells with PBS, 600g centrifuged for 10 minutes). Add an equal volume of reagent 1, boiled water bath for 5 minutes (wrap the sealing film to prevent explosion cover). After centrifugation at 12000 g for 10 minutes at 4℃e, the supernatant is taken and placed at 4 °C for testing. If the test cannot be completed temporarily, it can be stored at-80 °C (which can be stored for 3 days).

3. Cell sample

According to the proportion of cell number (10^6): reagent volume (mL) of 5~10 : 1 (it is recommended to add 1mL reagent 1 to 5 million cells), repeated freezing and thawing 2-3 times (it can be frozen in liquid nitrogen and dissolved in 37 °C water bath) or ice bath ultrasonic crushing cells (power 200w, ultrasonic 3s, interval 10s, repeat 30 times), 12000g centrifuged for 10 minutes, the supernatant is placed on the ice for testing. If the test cannot be completed temporarily, it can be stored at-80 °C (can be stored for 3 days).

II. Detection

1. Preheat spectrophotometer for 30 minutes, adjust the wavelength to 412 nm, set zero with distilled water.
2. Preparation of standards: aspirate 10mg/mL standard solution and dilute it with distilled water to 200μg/mL, 100μg/mL, 50μg/mL, 25μg/mL, 12.5μg/mL.
3. Operation table: Add the following reagents to the 1.5mL EP tube respectively.

Reagent（μL）	Test tube (T)	Standard tube (S)	Black tube (B)
Sample	100	-	-
Standard	-	100	-
Distilled water	-	-	100
Reagent II	700	700	700
Reagent III	200	200	200
After mixing and stewing at room temperature for 2 min, the absorbance at 412 nm of the test tube, standard tube and blank tube were recorded as A_T, A_S and A_B, respectively, $\Delta A=A_T-A_B$ and $\Delta A_S=A_S-A_B$. The standard curve and blank tube should be done only 1-2 times.			

III. Calculations

According to the concentration of the standard tube (x, μg/mL) and the absorbance ΔAs (y, ΔAs), a standard curve was established. According to the standard curve, ΔA (y, ΔA) was brought into the formula to calculate the sample concentration (x, μg/mL).

1) Protein concentration

GSH（μg /mg prot）=x ×V_{RV}÷（V_{RV}× Cpr）=x÷Cpr

2) Sample weight

GSH（μg /g weight）=x ×V_{RV}÷(V_{RV}÷ Vsv× W)=x÷W

3) Cell amount

GSH（μg/10^6 cell）=x×V_{RV}÷(V_{RV}÷Vsv×N)=x÷N

4) Solution volume

GSH（μg/mL）=2x

N: Cell amount, count by 10^6;

V_{SV}: Total supernatant volume, 1 mL;

V_{RV}: Supernatant volume added into the reaction system, 100 μL=0.1 mL;

W: Sample weight, g;

Cpr: Supernatant protein concentration, mg/mL;

2: The volume of plasma (blood cells) is diluted by one time.

Note:

1. The sample needs to be homogenized completely. If the test cannot be completed temporarily, it can be stored at -80℃ for 3 days.
2. If the GSH content in the sample is uncertain, Dilute the sample for several gradients before test.
3. Because reagent I contains protein precipitant, the supernatant cannot be used for protein concentration determination. If the protein content needs to be determined, take another tissue.
4. If the measured absorbance value exceeds the linear range absorbance value, you can increase the sample volume or dilute the sample before measurement.

Recent Product Citations

[1] FangzhouChen,YingZhao,Huizhao Chen. MicroRNA-98 reduces amyloid β-protein production and improves oxidative stress and mitochondrial dysfunction through the Notch signaling pathway via HEY2 in Alzheimer's disease mice. International Journal of Molecular Medicine.October 2018;91-102.(IF2.784)

[2] Ming Song,FangfangChen,YihuiLi,et al. rimetazidine restores the positive adaptation to exercise training by mitigating statin-induced skeletal muscle injury. Journal of Cachexia, Sarcopenia and Muscle. November 2017;(IF10.754)

[3] Hua Li,LanyingWang,Yanping Luo. Composition Analysis by UPLC-PDA-ESI (−)-HRMS and Antioxidant Activity Using Saccharomyces cerevisiae Model of Herbal Teas and Green Teas from Hainan. Molecules. October 2018;(IF3.06)

[4] OuYang Q, Tao N, Zhang M. A damaged oxidative phosphorylation mechanism is involved

in the antifungal activity of citral against Penicillium digitatum[J]. Frontiers in microbiology, 2018, 9: 239.

[5] Chen Z Y, Wang Y T, Pan X B, et al. Amelioration of cold-induced oxidative stress by exogenous 24-epibrassinolide treatment in grapevine seedlings: Toward regulating the ascorbate–glutathione cycle[J].
Scientia horticulturae, 2019, 244: 379-387.

[6] GongSun X, Zhao Y Q, Jiang B, et al. Inhibition of MUC1-C regulates metabolism by AKT pathway in esophageal squamous cell carcinoma[J]. Journal of cellular physiology, 2019, 234(7): 12019-12028.

Reference:

[1] Alpert A J, Gilbert H F. Detection of oxidized and reduced glutathione with a recycling postcolumn reaction[J]. Analytical biochemistry, 1985, 144(2): 553-562.

[2] Owens C W I, Belcher R V. A colorimetric micro-method for the determination of glutathione[J]. Biochemical Journal, 1965, 94(3): 705.

Related products：

BC1180/ BC1185	Oxidized Glutathione（GSSG）Assay Kit
BC1190/ BC1195	Glutathione Peroxidase Assay Kit
BC1150/ BC1155	Oxidized Thioredoxin Reductase (TrxR) Assay Kit
BC1210/ BC1215	γ-glutamate-cysteine ligase (GCL) Assay Kit