Reduced Glutathione (GSH) Content Assay Kit

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

Operation Equipment: Spectrophotometer/Microplate Reader

Catalog Number: NA0540

Size: 100T/96S

Components:

Reagent I: 100 mL×1. Store at 4℃.

Reagent II: 20 mL×1. Store at 4℃.

Reagent III: 8 mL×1. Store at 4℃, protect from light.

Standard: Powder 10 mg×1. Store at 4℃, protect from light.

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：3.763 μg/mL

Linear Range：12.5-400 μg/mL

Reagents and Equipment Required but Not Provided

Analytical balance, mortar/homogenizer, low temperature centrifuge, water bath, adjustable pipette, spectrophotometer/microplate reader, micro glass cuvette or 96 well flat-bottom plate and distilled water.

Procedure

I. Sample preparation

1. Tissue sample

Wash fresh tissues with PBS for twice, then add 0.1 g of animal/plant tissueinto homogenizer (the homogenizer has been rinsed with Reagent I and placed on ice before use). Add 1 mL of Reagent I (the proportion of tissue and Reagents can be kept constant), fully grinding on ice (using liquid nitrogen will have a better grinding effect). Centrifuge at 8000 ×g for 10 minutes at 4℃, take the supernatant and place it at 4℃ for test. (If the test cannot be completed temporarily, the supernatant can be stored at -80℃ for 10 days.)

2. Blood sample

Plasma: Sample is centrifuged at 600 ×g for 10 minutes at 4℃. Absorbing the upper plasma into another tube with adding the same volume Reagent I. Centrifuge at 8000 ×g for 10 minutes at 4℃, take

the supernatant and place it at 4℃for test. (If the test cannot be completed temporarily, the supernatant can be stored at -80℃ for 10 days.)

Blood cell: Sample is centrifuged at 600 ×g for 10 minutes at 4℃. Discarding the upper plasma, wash with three times volume of PBS for 3 times (re-suspendblood cell with PBS, centrifuge at 600 ×g for 10 minutes), add equal volume of Reagent I. After mixing, it is placed at 4℃ for 10 minutes. Centrifuge at 8000 ×g for 10 minutes, take the supernatant and place it at 4℃ for test. (If the test cannot be completed temporarily, the supernatant can be stored at -80℃ for 10 days.)

3. Cell sample

Harvesting cell should not less than 10^6,then wash it with PBS for twice (re-suspend cell with PBS, centrifuge at 600 ×g for 10 minutes). The volume of Reagent I added is three times the volume of cell precipitation to re-suspend the cells. Repeated freezing and thawing 2-3 times (It is suggested that frozen in liquid nitrogen, dissolved in 37℃ water bath). Centrifuge at 8000 ×g for 10 minutes, take the supernatant and place it at 4℃ for test. (If the test cannot be completed temporarily, the supernatant can be stored at -80℃ for 10 days.)

II. Procedure

1. Preheat spectrophotometer/microplate reader for 30 minutes, adjust the wavelength to 412 nm, set zero with distilled water.
2. Preheat ReagentII in 37℃ (mammal cell) or 25℃ (other species) water bath for 30 minutes.
3. Blank tube determination: take micro glass cuvette, add 20 μLof distilled water, 140 μL of Reagent II, 40 μL of Reagent III in turn, mix well, place for 2 minutes, and measure 412 nm absorbance A_B.
4. Making standard curve

Weigh 1 mg of standard and dissolve it with 1 mL of distilled water to obtain the concentration of 1 mg/mL. Take the appropriate solution to prepare the standards with the concentration of 200 μg/mL, 100 μg/mL, 50 μg/mL, 25 μg/mL and 12.5 μg/mL(Dilute Reagent I ten times before diluting the standard solution).

Take a 1.5 mL EP tube and add 20 μL of standard, 140 μL of Reagent II and 40 μL of Reagent III in turn. After each tube is evenly mixed, it is allowed to stand for 2 minutes. Measure the absorbance at 412 nm, and absorbance minus A_B as abscissa. Make the standard curve according to the absorbance (x) and concentration (y, μg/mL).

5. Sample tube test: take micro glass cuvette, add 20 μL of sample, 140 μL of Reagent II, 40 μL of Reagent III in turn, mix well, and then stand for 2 minutes to test the absorbance $A_T$at 412 nm, $\Delta A = A_T - A_B$.
6. The operation of microplate reader is the same as that of the spectrophotometer, and the operation is as fast as possible.

III. Calculations

According to the standard curve, take sample ΔA into the formula(x), and calculate the sample concentration y (μg/mL).

1) Protein concentration

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

2) Sample weight

GSH (μg/g)=y×V_{RV}÷(V_{RV}÷V_{SV}×W)= y÷W

3) Cell amount

GSH (μg/10^4cell)=y×V_{RV}÷(V_{RV}÷V_{SV}×N)= y÷N

4) Solution volume

GSH (μg/mL)= 2y

N: Cell amount, 10^6;

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

V_{RV}: Supernatant volume added into the reaction system, 20 μL=0.02 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℃.
2. Standard: Reduced glutathione is prepared when the solution will be used.
3. If the GSH content in the sample is uncertain,Dilute the sample for several gradients before test.
4. 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.

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：

NA0780/ NA0539 Oxidized Glutathione（GSSG）Assay Kit
NA0779/ NA0538 Glutathione Peroxidase Assay Kit
NA0783/ NA0542 Oxidized Thioredoxin Reductase (TrxR) Assay Kit
NA0778/ NA0537 -glutamate-cysteine ligase (GCL) Assay Kit