Reduced Glutathione (GSH) Content Assay Kit

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

Operation Equipment: Spectrophotometer/Microplate Reader

Catalog Number: AK0477-100T-96S

Components:

Reagent I: Liquid 110 mL×1. Store at 2-8℃.

Reagent II: Liquid 20 mL×1. Store at 2-8℃.

Reagent III: Liquid 5 mL×1. Store at 2-8℃, protect from light.

Standard: Powder×1. Store at 2-8℃. 10mg of reduced glutathione (GSH). Add 1mL distilled water to dissolve before use. The reagent can be stored at 2-8°C 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：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.

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 room temperature, 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 room temperature, 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).

Procedure

1. Preheat spectrophotometer/microplate reader for 30 minutes, adjust the wavelength to 412 nm, spectrophotometer set zero with distilled water.
2. Preparation of standards: aspirate 10mg/mL standard solution and dilute it with distilled water to 300μg/mL、200μg/mL、100μg/mL、50μg/mL、25μg/mL.
3. Operation table: Add the following reagents to the 1.5mL EP tube/96 well plate respectively.

Reagent（μL）	Test tube (T)	Standard tube (S)	Black tube (B)
Sample	20	-	-
Standard	-	20	-
Distilled water	-	-	20
Reagent II	140	140	140
Reagent III	40	40	40
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.			

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）$=x \times V_{RV} \div (V_{RV} \times Cpr) = x \div Cpr$

2) Sample weight

GSH（μg /g）$=x \times V_{RV} \div (V_{RV} \div V_{sv} \times W) = x \div W$

3) Cell amount

GSH（μg/10^6 cell）$=x \times V_{RV} \div (V_{RV} \div V_{sv} \times N) = x \div N$

4) Solution volume

GSH（μg/mL）=2x

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°C.
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] Wei M, Bai J, Shen X, Lou K, Gao Y, Lv R, Wang P, Liu X, Zhang G. Glutathione-Exhausting Nanoprobes for NIR-II Fluorescence Imaging-Guided Surgery and Boosting Radiation Therapy Efficacy via Ferroptosis in Breast Cancer. ACS Nano. 2023 Jun 27;17(12):11345-11361. doi: 10.1021/acsnano.3c00350. Epub 2023 Jun 5. PMID: 37272787; PMCID: PMC10311599.

[2] Li K, Xu K, He Y, Yang Y, Tan M, Mao Y, Zou Y, Feng Q, Luo Z, Cai K. Oxygen Self-Generating Nanoreactor Mediated Ferroptosis Activation and Immunotherapy in Triple-Negative Breast Cancer. ACS Nano. 2023 Mar 14;17(5):4667-4687. doi: 10.1021/acsnano.2c10893. Epub 2023 Mar 2. PMID: 36861638.

[3] Yang L, Zhang D, Li W, Lin H, Ding C, Liu Q, Wang L, Li Z, Mei L, Chen H, Zhao Y, Zeng X. Biofilm microenvironment triggered self-enhancing photodynamic immunomodulatory microneedle for diabetic wound therapy. Nat Commun. 2023 Nov 23;14(1):7658. doi: 10.1038/s41467-023-43067-8. PMID: 37996471; PMCID: PMC10667311.

[4] Li K, Lin C, Li M, Xu K, He Y, Mao Y, Lu L, Geng W, Li X, Luo Z, Cai K. Multienzyme-like Reactivity Cooperatively Impairs Glutathione Peroxidase 4 and Ferroptosis Suppressor Protein 1 Pathways in Triple-Negative Breast Cancer for Sensitized Ferroptosis Therapy. ACS Nano. 2022 Feb 22;16(2):2381-2398. doi: 10.1021/acsnano.1c08664. Epub 2022 Jan 18. PMID: 35041395.

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.