Oxidized Glutathione (GSSG) Content Assay Kit

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

Operation Equipment: Spectrophotometer/microplate reader

Catalog Number: BC1185

Size: 100T/96S

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 110 mL×1	2-8℃
Reagent II	Liquid 130 μL×1	2-8℃
Reagent III	Liquid 20 mL×1	2-8℃
Reagent IV	Liquid 2.5 mL×1	2-8℃
Reagent V	Powder×1	2-8℃
Reagent VI	Liquid 12.5 μL×1	2-8℃
Standard	Powder×1	2-8℃

Solution Preparation:

1. Reagent II: Toxic volatile reagent, steps involving this reagent are recommended to be performed in a fume hood.
2. Reagent V: Dissolve with 2.5 mL of distilled water when the solution will be used, then split into smaller packages, store at -20℃.
3. Reagent VI working liquid: First centrifuge the liquid in reagent 6 to the bottom and then use the pipette to blow and mix. Before clinical use, the reagent was prepared according to the proportion of Reagent VI: distilled water =1μL: 20μL (21μL, about 10T) according to the number of samples.
4. Standard: 10mg oxidized glutathione. Before use, add 1 mL distilled water, dissolve fully at a concentration of 10 mg/mL, and store for 4 weeks at 2-8℃.

Product Description

Oxidized Glutathione(GSSG) is an oxidized form of glutathione (GSH), also known as dithioneglutathione, formed by the oxidation of two molecules of glutathione. GSSG is reduced to GSH by glutathione reductase, so most of the body is in the reduced form. The determination of GSH and GSSG content and ratio of GSH/GSSG in cells can reflect the redox status of cells. This kit utilizes reaction of glutathione and 5, 5'-dithiobis-2-nitrobenoic acid (DTNB) to produce 5-thio-2- nitrobenzoic acid. 5-thio-2-nitrobenzoic acid has the largest absorption at wavelength of 412nm, and 2-Vinylpyridine inhibit reduced glutathione in the original of samples, and then using glutathione reductase to reduce GSSG to GSH, determining the content of Oxidized Glutathione.

Technical Specifications

Minimum Detection Limit：3.211 μg/mL

Linear Range：3.9-125 μg/mL

Reagents and Equipment Required but Not Provided.

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

Procedure

I. Sample preparation

1. Tissue sample

Wash fresh tissues with PBS for twice, then add 0.1 g of sample into 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 and 4℃ for 10 minutes, take the supernatant and place it at 4℃ for test. (The supernatant can be stored at -80℃ for 3 days.)

2. Blood sample

Plasma: Sample is centrifuged at 600 ×g and 4℃ for 10 minutes. Absorbing the upper plasma into another tube add with same volume Reagent I. Centrifuge at 8000 ×g and 4℃ for 10 minutes, take the supernatant and place it at 4℃ for test. (The Supernatant can be stored at -80℃ for 10 days.)

Blood cell: Sample is centrifuged at 600 ×g and 4℃ for 10 minutes. Discarding the upper plasma, wash with treble volume of PBS for 3 times (mix blood 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. (The supernatant can be stored at -80℃ for 3 days.)

3. Cell sample

Harvesting cell should not less than 10^6, then wash with PBS for twice (mix cell with PBS centrifuge at 600 ×g for 10 minutes), mix precipitated cell with the volume of PBS for 3 times. Repeat freezing and thawing 2-3 times (suggest frozen in liquid nitrogen, dissolved in 37℃ water bath) or ultrasonic (placed on ice, 200W, work time 3s, interval 10s, repeat for 30 times). Centrifuge at 8000 ×g for 10 minutes, take the supernatant and place it at 4℃ for test. (The supernatant can be stored at -80℃ for 3 days.)

II. Procedure

1. Preheat spectrophotometer/microplate reader for 30 minutes, adjust the wavelength to 412 nm, set the counter to zero with distilled water.
2. Preheat Reagent II in water bath for 30 minutes at 37℃.
3. The standard dilution: dissolve standard with 1 mL of distilled water (4℃) to concentration of 10 mg/mL. Take suitable solution to prepare the standard of concentration of 125μg/mL 、62.5μg/mL、31.25μg/mL、15.625μg/mL、7.8125μg/mL、3.90625μg/mL and 0μg/mL with distilled water.

4. Add 20 μL of diluted standard or sample to 0.5 mL centrifuge tube, add 1 μL of Reagent II, incubate at 37 ℃ for 30 minutes after mixing.

5. Operation table

Reagent Name（μL）	Test tube (A_T)	Standard tube (A_S)	blank tube (A_B)
Sample	20	-	-
Standard	-	20	-
H_2O	-	-	20
Reagent II	1	1	1
React at 37℃ for 30 mins			
Reagent III	140	140	140
Reagent IV	20	20	20
Reagent V	20	20	20
Reagent VI	2	2	2
Add Reagent VI at the same time began timing, rapid mixing, determination of 412 nm at 30s and 150s light absorption $A1_T$ ($A1_S$, $A1_B$) and $A2_T$ ($A2_S$, $A2_B$), calculation of $\Delta A_T = A2_T - A1_T$, $\Delta A_S = A2_S - A1_S$, $\Delta A_B = A2B - A1_B$, blank tube and standard curve only need to do 1-2 times.			

III. Calculations

1) According to the concentration of the standard tube (x, μg/mL) and the absorbance ($\Delta As-\Delta A_B$) (y, $\Delta As-\Delta A_B$), a standard curve is established. According to the standard curve, ($\Delta A_T-\Delta A_B$) is brought into the formula to calculate the sample concentration (x, μg/mL).
2) Calculation by protein concentration
   GSSH (μg / mg prot)= x×Vsv÷Vsv÷Cpr =x÷Cpr
3) Calculation by sample weight
   GSSH (μg /g)= x×Vsv÷(Vsv÷Vev×W)= x÷W
4) Calculation by cell amount
   GSSH (μg /10^6 cell)= x×Vsv÷(Vsv÷Vev×N)= x÷N
5) Calculation by solution volume
   GSSH (μg / mL)= 2x

N: Cell amount,10^6 cells as a unit;
Vsv: The volume of supernatant was added into the reaction system, 20 μL=0.02 mL;
W: Sample weight, g;
Cpr: Supernatant protein concentration, mg/mL.
Vev: Total volume of pre-treated supernatant, 1mL.

Notes:

1. The sample needs to be homogenized completely. If the test cannot be completed temporarily, it

can be stored at -80℃.

2. If the content of GSSG content in the sample is uncertain, several gradients can be diluted before

measurement.

3. This method uses the enzymatic reaction rate to calculate the substrate concentration and complete readings as accurately as possible at 30 and 150 seconds.

4. Reagent I contained protein precipitant, the supernatant could not be used for protein concentration determination. If the protein content needs to be determined, take another tissue.

Recent Product citations

[1] 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)

[2] 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)

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:

BC1170/ BC1175	Reduced Glutathione（GSH）Assay Kit
BC1190/ BC1195	Glutathione Peroxidase Assay Kit
BC0350/ BC0355	Glutathione S-transferase (GST) Activity Assay Kit
BC1160/ BC1165	Glutathione Reductase (GR) Assay Kit