Oxidized Glutathione (GSSG) Content Assay Kit

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

Operation Equipment: Spectrophotometer

Catalog Number: BC1180

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 170 μL×1	2-8℃
Reagent III	Liquid 60 mL×1	2-8℃
Reagent IV	Liquid 8 mL×1	2-8℃
Reagent V	Powder×1	2-8℃
Reagent VI	Liquid 40 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 8 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 IV 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 2T) 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 dithione glutathione, which 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 412 nm, 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

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Minimum Detection Limit：1.369μg/mL

Linear Range：1.5625-50μg/mL

Reagents and Equipment Required but Not Provided

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

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 12000 ×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 1200 ×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.)

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 12000 ×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 5×10^6, then wash with PBS for twice (mix cell with PBS, centrifuge at 600 ×g for 10 minutes), mix precipitated cell with 1mL Reagent I. Repeated freezing, thawing 2-3 times (suggest frozen in liquid nitrogen, dissolved in 37℃ water bath) or ultrasonic in ice bath (200w, ultrasound 3s, interval 10s, repeat 30 times). Centrifuge at 12000×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 for 30 minutes, adjust the wavelength to 412 nm, set the counter to zero with distilled water.
2. Preheat Reagent III 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 with water of concentration of 50μg/mL、25μg/mL、12.5μg/mL、6.25μg/mL、3.125μg/mL、1.5625μg/mL.
4. Operation table:

Reagent Name（μL）	Test Tube (A_T)	Standard Tube (A_S)	Blank Tube (A_B)
Sample	100	-	-
Standard	-	100	-
Distilled water	-	-	100
Reagent II	2	2	2
React 37℃ for 30 mins			
Reagent III	700	700	700
Reagent IV	100	100	100
Reagent V	100	100	100
Reagent VI	10	10	10
Add reagent VI rapid mixing and at the same time begin timing, determination of 412 nm at 30 s and 150 s light absorption A1 ($A1_T$, $A1_S$, $A1_B$) and A2 ($A2_T$, $A2_S$, $A2_B$), calculation of $\Delta A_T = A2_T - A1_T$, $\Delta A_S = A2_S - A1_S$, $\Delta A_B = A2_B - A1_B$, blank tube and standard curve only need to do 1-2 times.			

III. Calculations

1) The standard curve was drawn with the concentration of standard tube (μg / mL) as x and ($\Delta A_S - \Delta A_B$) as y. According to the standard curve, ($\Delta A_T - \Delta A_B$) is brought into the formula to calculate the sample concentration (μg / mL).Protein concentration
2) Protein concentration
   GSSH (μg/mg prot)=x×Vrv÷Vrv÷Cpr =x÷Cpr
3) Sample weight
   GSSH (μg/g)= x×Vrv÷(Vrv÷Vsv×W)= x÷W
4) Cell amount
   GSSH (μg/10^4cell)= x×Vrv÷(Vrv÷Vsv×N)= x÷N
5) Solution volume
   GSSH (μg/mL)= 2x

N: Cell amount, 10^6 as a unit;
Vsv: Total supernatant volume, 1 mL;
Vrv: Supernatant volume added into the reaction system, 100 μL=0.1 mL;
W: Sample weight, g;
Cpr: Supernatant protein concentration, mg/mL.

Note:

1. The sample needs to behomogenized completely. If the test cannot be completed temporarily, it can be stored at -80℃.
2. If the GSSG content in the sample is uncertain,dilute the sample for several gradients before test.

3. This method uses the enzymatic reaction rate to calculate the substrate concentration and complete readings as accurately as possible at 30s and 150s.
4. 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] 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)
[2] Chen Z Y, Wang Y T, Pan X B, et al. Amelioration of cold-induced oxidative stress by exogenous 24-epibrassinolidetreatment in grapevine seedlings:Toward regulating the ascorbate–glutathione cycle[J].Scientia horticulturae,2019,244:379-387.

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