Oxidized Glutathione (GSSG) Content Assay Kit

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

Operation Equipment: Spectrophotometer

Catalog Number: NA0780

Size: 50T/48S

Components:

Reagent I: 50 mL×1. Store at 4℃.

Reagent II: 170 μL×1. Store at 4℃.

Reagent III: 60 mL×1. Store at 4℃.

Reagent IV: 8 mL×1. Store at 4℃.

Reagent V: Powder×1. Storage at 4℃. Dissolve with 8 mL of distilled waterwhen the solution will be used, then split into smaller packages, store at -20℃.

Reagent VI: 40 μL×1. Store at 4℃. Prepare Reagent VI and distilled water according to the sample size at the ratio of 1:20 (V: V) before use.

Standard: 10 mg×1. Store at 4℃.

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

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 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 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.)

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 10 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.Repeated freezing and thawing 2-3 times (suggest 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. (The supernatant can be stored at -80℃ for 10 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 II in water bath: 37℃ (mammal cell) or 25℃ (other species).
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 50μg/mL、25μg/mL、12.5μg/mL、6.25μg/mL、3.125μg/mL、1.5625μg/mL and 0μg/mL (The diluent is a ten-fold diluted Reagent I).
4. Add 100 μL of diluted standard or sample to 1.5 mL centrifuge tube, add 2 μL of Reagent II,mix well, incubate at 37℃ for 30 minutes.
5. Make standard curve

After the incubation, add 700 μL of ReagentIII, 100 μL of Reagent IV, 100 μL of Reagent V, and 10 μL of Reagent VI to the standard tube in sequence. After rapid mixing, the light absorption A1 and A2 of 30 s and 150 s respectively were measured at 412 nm. Absorbance (A2-A1) is the abscissa (x) and concentration is the ordinate (y), making the standard curve.

6. Add 700 μL of Reagent III, 100 μL of Reagent IV, 100 μL of Reagent V, and 10 μL of Reagent VI to the sample tubes in sequence. After rapid mixing, the light absorption A1 and A2 of 30 s and 150 s respectively were measured at 412 nm, $\Delta A = A2 - A1$.

III. Calculations

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

1) Protein concentration

GSSH (μg/mg prot)=y×Vrv÷Vrv÷Cpr =y÷Cpr

2) Sample weight

GSSH (μg/g)= y×Vrv÷(Vrv÷Vsv×W)= y÷W

3) Cell amount

GSSH (μg/10^4cell)= y×Vrv÷(Vrv÷Vsv×N)= y÷N

4) Solution volume

GSSH (μg/mL)= 2y

N: Cell amount, 10^6;

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：

NA0781/ NA0540	Reduced Glutathione（GSH）Assay Kit
NA0779/ NA0538	Glutathione Peroxidase Assay Kit
NA0839/ NA0597	Glutathione S-transferase(GST) Activity Assay Kit
NA0782/ NA0541	Glutathione Reductase (GR) Assay Kit