(FOR RESEARCH USE ONLY. DO NOT USE IT IN CLINICAL DIAGNOSIS!)

Total Glutathione /Oxidized Glutathione (T-GSH/GSSG) Assay Kit

Catalog No.: BC00026

Size: 100T

Please read the instructions carefully before use. If you have any questions or need further help during experiment, please don't hesitate to contact us through the following methods:

✉ Email (Sale) order@enkilife.com

✉ Email (Techsupport) techsupport@enkilife.com

☎Tel: 0086-27-87002838

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Shelf life: Please refer to the label on the outer package.

Techsupport: In order to provide you with better service, please inform us the lot number on the label of the outer package.

Basic Information

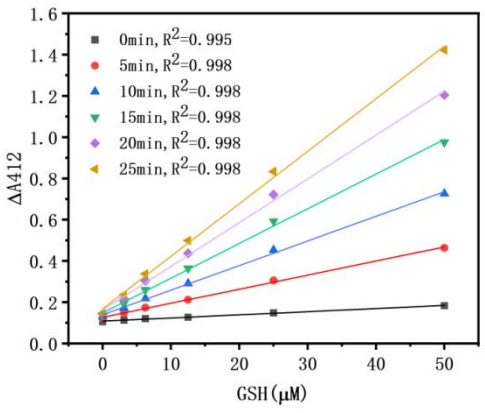
Product Name	Total Glutathione /Oxidized Glutathione (T-GSH/GSSG) Assay Kit
Detection Method	Colorimetric
Sample Type	Tissue, cells, plasma
Assay Type	Quantitative
Detection Instrument	Microplatereader(405-414nm,optimal detection wavelength 412nm)
Range	1-50μM
Sensitivity	0.1436μM

Product Introduction

Glutathione is a small peptide composed of three amino acid residues. Its full name is glutamyl-cysteinyl-glycine, Since the thiol (SH) on cysteine is the active group of glutathione, it is often abbreviated as G-SH or GSH. Glutathione includes two forms: reduced glutathione (commonly known as GSH) and oxidized glutathione (oxidized glutathione disulfide). Since oxidized glutathione is formed by the dehydrogenation of two GSH groups, it is often abbreviated as GSSG or GSSG. Reduced glutathione is the main source of thiol groups in most living cells. It plays an important role in maintaining the proper redox state of thiol groups in proteins and is a key antioxidant in animal cells. Usually 90-95% of total glutathione is reduced glutathione.

Product Features

● This kit provides protein removal reagent S, which can more accurately determine the amount of total glutathione in samples containing protein. The detection limit of this kit is 1μM. The actual measurement effect of the standard product is shown in the figure below:



Principle

Glutathione reductase can reduce oxidized glutathione (GSSG) to generate reduced glutathione (GSH), and GSH can react with the chromogenic substrate DTNB to produce yellow TNB and GSSG, and the amount of TNB generated can be detected by measuring A412. When the reaction system is properly set up and the two reactions are combined, the total glutathione (GSSG+GSH) is equivalent to a rate-limiting factor for color production, and the amount of total glutathione determines the amount of yellow TNB formed. Therefore, the amount of total glutathione can be calculated by measuring A412. The specific reaction principle of this kit is as follows:

$$2GSH + DTNB \rightarrow GSSG + 2TNB$$

$$NADPH + H^+ + GSSG \xrightarrow{GR} NADP^+ + 2GSH$$

$$DTNB + H^+ + NADPH \xrightarrow[GSSG/GSH]{GR} 2TNB + NADP^+$$

Components

Serial number	Components	Size(100T)	Storage
Reagent 1	Total Glutathione Assay Buffer	60ml	-20℃
Reagent 2	Glutathione reductase	150μl	-20℃
Reagent 3	Reduced Glutathione (GSH)	4.5mg	-20℃, prepare into solution,store at -20ºC after aliquoting.
Reagent 4	DTNB	4.5mg	-20℃, prepare into solution, store at -20ºC after aliquoting.
Reagent 5	Protein Removal Reagent S	0.4g	-20℃, prepare solution and store at 2-8 ºC.
Reagent 6	NADPH	4mg	At -20℃, NADPH is dissolved and packaged,stored at -70ºC. It can be stored at 2-8 ºC for one day. After being stored at -20℃ for one week, NADPH will degrade by more than 10%.
Reagent 7	DMSO	1.5ml	-20℃
Consumable 1	Microplate(96 wells)	1 plate	RT
Consumable 2	Plate Sealer	2 pieces	RT

Storage

The unopened kit can be stored at -20℃ for 12 months.

Experimential Preparation

- Sample processing

1. Preparation of tissue samples. Take the tissue and freeze it with liquid nitrogen, then grind it into powder. For every 10 mg of the ground tissue powder, add 30μl of Protein Removal Reagent S solution and vortex thoroughly. Then add 70μl of Protein Removal Reagent S solution and homogenize thoroughly with a glass homogenizer (for tissues that are easier to homogenize, you can directly add an appropriate amount of Protein Removal Reagent S solution for homogenization without liquid nitrogen freezing). After standing at 4ºC for 10 minutes, centrifuge at 10,000 x g for 10 minutes at 4ºC, and take the supernatant for the determination of total glutathione. The sample needs to be temporarily stored at 4ºC. Samples that are not measured immediately can be stored at -70ºC, but should not exceed 10 days. For the processed tissue samples, it is usually necessary to dilute them appropriately with Protein Removal Reagent S solution before determination. The dilution multiple is usually 5-20 times.
2. Preparation of cell samples. Try to use fresh cells for measurement instead of frozen cells. Wash cells once with PBS, collect cells by centrifugation, and aspirate the supernatant. Add 3 times the volume of the cell pellet, i.e. if the cell pellet is 10 μl, add 30 μl of the Protein Removal Reagent S solution, and vortex thoroughly. (The volume of the cell pellet can be estimated based on the weight of the cell pellet. Weigh the centrifuge tube before and after collecting the cells, so that the weight of the cell pellet can be calculated. The volume of 10mg of cell pellet can be roughly regarded as 10μl.) Then freeze and thaw the sample twice quickly using liquid nitrogen and a 37°C water bath. Place at 4°C or in an ice bath for 5 minutes. Centrifuge at 10,000 x g for 10 minutes at 4°C. Take the supernatant for the determination of total glutathione. The sample needs to be temporarily stored at 4°C. Samples that are not immediately measured can be stored at -70°C, but should not exceed 10 days. The treated cell samples usually need to be appropriately diluted with protein removal reagent S solution before measurement, and the dilution multiple can be as high as 20 times.
3. Preparation of red blood cell or plasma samples. Please use fresh blood for measurement. Centrifuge at 600 x g for 10 minutes. The precipitate is red blood cells and the supernatant is plasma. For red blood cells, wash twice with PBS. Take about 50 μl of red blood cell precipitate or plasma, add 50μl of Protein Removal Reagent S solution, and vortex thoroughly. Place at 4ºC or on ice for 10 minutes. Centrifuge at 10,000 x g for 10 minutes at 4ºC. Take the supernatant for total glutathione measurement. The sample needs to be temporarily stored at 4ºC. Samples that are not measured immediately can be stored at -70ºC, but should not exceed 10 days. For the processed red blood cell sample, it is necessary to dilute it 10 times with Protein Removal Reagent S solution before subsequent measurement. For plasma samples, 10μl should be directly taken for measurement.
4. For some samples with extremely low glutathione content, they can be concentrated

by freeze drying before measurement.

- Preparation of the assay kit

1. Preparation of GSH stock solution: Add 1.5 ml of Milli-Q grade pure water to 4.5 mg of GSH provided in this kit, dissolve and mix to obtain GSH stock solution with a concentration of 10 mM. Except for the portion to be used immediately, the remaining GSH stock solution should be appropriately divided and stored at -20°C.
2. Preparation of DTNB stock solution: Add 1.5 ml of DMSO provided in this kit to 4.5 mg of DTNB provided in this kit, dissolve and mix well to obtain the DTNB stock solution. Except for the portion to be used immediately, the remaining DTNB stock solution should be appropriately divided and stored at -20°C in the dark.
3. Preparation of Protein Removal Reagent S Solution: Add 8 ml of Milli-Q grade water to 0.4g of Protein Removal Reagent S provided in this kit to prepare 8 ml of 5% aqueous solution. Store at 4ºC.
4. Preparation of NADPH stock solution (40 mg/ml): Add 100 µl of Milli-Q grade pure water to the 4 mg NADPH provided in this kit, dissolve and mix to obtain the NADPH stock solution. Except for the portion to be used immediately, the remaining NADPH stock solution should be appropriately aliquoted and stored at -70°C.
5. Preparation of 5-fold diluted glutathione reductase: Take 50 µl of glutathione reductase,add 200 µl of total glutathione detection buffer, mix well, and you will get 5-fold diluted glutathione reductase.
6. Preparation of total glutathione detection working solution: According to the number of samples to be tested, refer to the table below to prepare an appropriate amount of total glutathione detection working solution. The total glutathione detection working solution is obtained by mixing the three reagents in the table in proportion.

	1 sample	10 samples	20 samples
5-fold dilution of glutathione reductase	6.6 µl	66 µl	132 µl
DTNB stock solution	6.6 µl	66 µl	132 µl
Total Glutathione Assay Buffer	150 µl	1.5 ml	3 ml

7. Preparation of 0.5mg/ml NADPH: Take 10 µl of NADPH stock solution, add 790µl of total glutathione detection buffer, and mix well to obtain 0.5mg/ml NADPH. 50µl of 0.5mg/ml NADPH are required for each sample tested.
8. Preparation of standard: Dilute 10mM GSH stock solution with total glutathione detection buffer to 50µM GSH solution. Then dilute to 25 , 12.5 , 6.25 , 3.125 µM GSH solution in half dilution method. Take 50, 25 , 25 , 12.5 , 6.25 , 3.125 , 0µM GSH solution for standard curve.

Operation process

1. Refer to the table below, use a 96-well plate, add samples or standards in sequence, mix well. Add 150 µl of total glutathione detection working solution, mix well, and incubate

at 25°C or room temperature for 5 minutes.

	Blank	Standard curve	sample
Sample or standard solution	0 μl	10 μl	x μl
Protein Removal Reagent S Solution	10 μl	0 μl	10-x μl
Total glutathione assay working solution	150 μl	150 μl	150 μl
Incubate at 25 ° C or room temperature	5 min	5 min	5 min
0.5mg/ml NADPH	50 μl	50 μl	50 μl

2. Add 50 μl of 0.5 mg/ml NADPH solution to each well and mix well.
3. Immediately measure A412 using an appropriate microplate reader or micro-volume UV spectrophotometer, measuring once every 5 minutes or measuring in real time for a total of 25 minutes, and obtaining 5 data. (Note: To simplify the experimental steps, A412 can be measured only once 25 minutes after adding the NADPH solution and mixing). If the instrument can be set to a temperature, set the temperature to 25ºC, otherwise measure at room temperature. If the microplate reader cannot measure A412, the absorbance in the range of 405-414nm can be measured. If the standard curve is good, but the absorbance of the sample is relatively low, the incubation time can be extended to 30-60 minutes. The absorbance of the standard and sample will increase linearly with time within a certain range.

Result calculation

1. Single-point determination method: that is, the absorbance is measured only once after 25 minutes (or 30-60 minutes) of reaction. A standard curve is made based on the different absorbances measured by standards of different concentrations. The total glutathione content can be calculated by comparing the sample with the standard curve. The actual calculated total glutathione content is equivalent to multiplying the content of oxidized glutathione by 2 and adding the content of reduced glutathione. The single-point method is relatively convenient, while the kinetic method is relatively accurate.
2. Kinetic determination method: First, calculate ΔA412/min based on the absorbance values measured at different time points. Then, make a standard curve with the concentration of the standard as the horizontal axis and ΔA412/min as the vertical axis. Based on the ΔA412/min of the sample, the total glutathione content in the sample during the determination can be calculated by comparing it with the standard curve.
3. At the same time, according to the dilution multiple of the sample and the initial sample usage, the total glutathione content per milligram of tissue or cell can be calculated. For cell samples, the protein content of the cell sample can also be calculated by measuring the protein concentration after taking a certain number of cells for lysis based on the initial

number of cells used, and finally calculating the total glutathione content per milligram of protein.

Notes

1. This kit involves redox reaction, and all oxidants or reducing agents will interfere with the determination of this kit. In particular, reagents containing thiol groups such as DTT and mercaptoethanol will seriously interfere with the determination of this kit, so please try to avoid them.
2. The reaction temperature and reaction time must be strictly controlled, otherwise a standard curve will need to be made each time.
3. NADPH are not very stable, so please strictly follow the subsequent instructions to prevent inactivation.
4. DMSO will solidify at low temperatures such as 4ºC or ice bath. It can be incubated in a 20-25ºC water bath for a while until it is completely melted before use.
5. This product is for Research Use Only, and shall not be used for clinical diagnosis or treatment, food or medicine, or stored in ordinary residences.