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

Reduced Glutathione & Oxidized Glutathione (GSH & GSSG) Assay Kit

Catalog No.: BC00027

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

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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	Reduced Glutathione & Oxidized Glutathione (GSH & GSSG) Assay Kit
Detection Method	Colorimetric
Sample Type	Tissue, Cells, Plasma
Assay Type	Quantitative
Detection Instrument	Microplate reader (405-414nm, optimal detection wavelength 412 nm)
Range	0.5-20μM
Sensitivity	0.0494μM

Product Introduction

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

Product Features

● The detection limit of this kit is 0.5μM, and it can detect the content of GSH and GSSG in animal tissues, plasma, red blood cells, cultured cells or other appropriate samples.
● This kit provides protein removal reagent M, which can more accurately measure samples containing protein.

Principle

GSSG is reduced to GSH by glutathione reductase, and GSH can react with the chromogenic substrate DTNB to produce yellow TNB and GSSG. When the reaction system is properly prepared and the two reactions are combined, the total glutathione (GSSG+GSH) is equivalent to a rate-limiting factor in 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 the absorbance value at 412nm. Use appropriate reagents to first remove GSH from the sample, and then use the above reaction principle to determine the GSSG content. The GSH content can be calculated by deducting the GSSG content from the total glutathione (GSSG+GSH).
The specific reaction principle of this kit is as follows:

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

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

The two reactions are combined:

$$NADPH + H^+ + DTNB \xrightarrow{GR} NADP^+ + 2TNB$$

Components

Serial number	Components	Size(100T)	Storage
Reagent 1	Total Glutathione Assay Buffer	60ml	-20℃
Reagent 2	Glutathione reductase	150μl	-20℃
Reagent 3	Oxidized Glutathione (GSSG)	5mg	-20℃, GSSG is prepared into solution, packaged, and stored at -20ºC for at least 3 months
Reagent 4	DTNB	4.5mg	-20℃, after DTNB is dissolved in DMSO, it is packaged and stored at -20ºC for at least 3 months.
Reagent 5	Protein Removal Reagent M	1g	-20℃, Protein Removal Reagent M can only be used on the same day after being prepared into solution.
Reagent 6	NADPH	4mg	-20°C, dissolve NADPH, aliquot, and store at -70°C.
Reagent 7	DMSO	1.5ml	-20℃
Reagent 8	GSH Scavenging reagents	500μl	-20℃, GSH scavenging reagent solution must be freshly prepared for use.
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.

Experimental 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 30 μl of protein removal reagent M solution and vortex thoroughly. Then add 70 μl of Protein Removal Reagent M 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 M solution for homogenization without liquid nitrogen quick freezing). After standing at 4ºC for 10 minutes, centrifuge at 10,000 x g at 4ºC for 10 minutes, 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 M 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 10μl, add 30μl of the protein removal reagent M 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 10 mg of cell pellet can be roughly regarded as 10μl.) Then freeze and thaw the sample twice quickly using liquid nitrogen and a 37ºC water bath. Place at 4ºC or in an ice bath for 5 minutes. Centrifuge at 10,000 x g for 10 minutes at 4ºC. Take the supernatant for the determination of total glutathione. The sample needs to be temporarily stored at 4ºC. Samples that are not immediately measured can be stored at -70ºC, but should not exceed 10 days. The treated cell samples usually need to be appropriately diluted with protein removal reagent M 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 as much as possible. 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 50μl of red blood cell precipitate or plasma, add 50μl of protein removal reagent M solution, and vortex thoroughly. Place at 4ºC or in an ice bath 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 M solution before subsequent measurement. For plasma samples, 10 microliters

should be directly taken for measurement.

4. illustrate : For some samples with extremely low glutathione content, they can be concentrated by freeze drying before measurement.

5. **Preparation of samples for GSSG content testing** : Take part of the above prepared samples for total glutathione content testing, add GSH scavenging working solution at a ratio of 4μl of GSH scavenging reagent working solution per 100μl of sample, immediately vortex mix, and react at 25°C for 60 minutes . The above reaction can remove up to 50puM of GSH. If the GSH content in the sample is too high, it needs to be appropriately diluted before removing GSH. After the above treatment, it can be used for subsequent determination.

- Preparation of the assay kit

1. Preparation of GSSG stock solution: Add 816μl of Milli-Q grade pure water to the 5mg GSSG provided in this kit, dissolve and mix well to obtain the GSSG stock solution with a concentration of 10mM. Except for the portion to be used immediately, the remaining GSSG stock solution should be appropriately divided and stored at -20 º 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 -20ºC in the dark .

3. Preparation of Protein Removal Reagent M Solution: Weigh 0.2g of Protein Removal Reagent M and add 4ml of Total Glutathione Assay Buffer to prepare 4ml of 5% aqueous solution. Protein Removal Reagent M solution must be freshly prepared and used only on the same day.

4. Preparation of NADPH stock solution (40mg/ml): Add 100μl of Milli-Q grade pure water to the 4mg NADPH provided in this kit, dissolve and mix well to obtain the NADPH stock solution.Except for the portion to be used immediately, the remaining NADPH stock solution should be appropriately divided and stored at -70 º C.

5. Preparation of 5-fold diluted glutathione reductase: Take 50μl of glutathione reductase,add 200μl 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 10μl 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 GSH scavenging reagent working solution: Add 89.2 μl of anhydrous ethanol to 10.8μl of GSH scavenging reagent and mix immediately. GSH scavenging reagent working solution must also be prepared fresh each time.
9. Preparation of standards :
(1) the 10mM GSSG stock solution with total glutathione detection buffer to make a 20μM GSSG solution. Then dilute it to 10, 5, 2.5, and 1.25μM GSSG solutions in sequence. Take six points of 20, 10, 5, 2.5, and 1.25μM GSSG solutions to make a standard curve.
(2) When used to determine GSSG, each well of the standard curve needs to add 0.4 μl of GSH scavenging working solution to the total glutathione detection working solution (i.e., add GSH scavenging working solution at a ratio of 0.4 μl of GSH scavenging reagent working solution to every 150 μl of total glutathione detection working solution), and immediately vortex mix to keep the solution system consistent with the sample.

Operation process

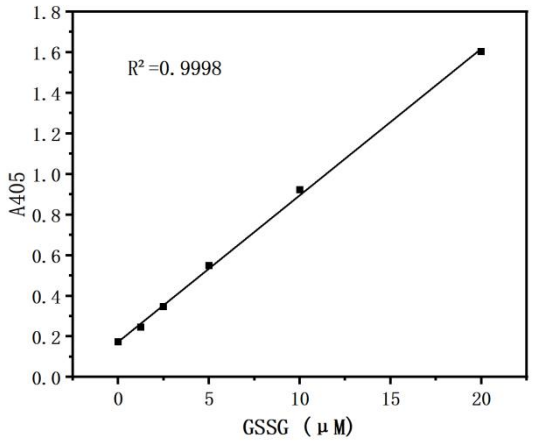
1. Refer to the table below, use a 96-well plate, add samples or standards in sequence, mix well. Add 150μl of total glutathione detection working solution, mix well, and incubate at 25ºC or room temperature for 5 minutes.

	Blank	Standard curve	Sample
Sample or standard	0 μl	10 μl	x μl
Protein Removal Reagent M 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 and mix well.
3. Immediately measure A412 with a microplate reader, measure once every 5 minutes or measure in real time, for a total of 25 minutes, and measure 5 data. **Note:** To simplify the experimental steps, you can measure A412 only once 25 minutes after adding the NADPH solution and mixing. If the instrument can set the temperature, set the temperature to 25ºC, otherwise measure at room temperature. If the microplate reader cannot measure A412, you can measure the absorbance in the range of 405-414nm. If the standard curve is good, but the absorbance of the sample is relatively low, you can extend the incubation time to 30-60 minutes. The absorbance of the standard and sample will increase linearly with time within a certain range.
Note : If the GSSG content is to be determined, the standard sample must also be subjected to the relevant operation of removing GSH in parallel to reduce the error. If the total glutathione content and GSSG content of the sample need to be determined at the

same time, since the detection systems of the two are different, separate standard curves must be made.

4. Reference figure of the measured effect of the standard product after adding NADPH solution and mixing for 25 minutes



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 (GSSG concentration calculated by the standard curve multiplied by 2) or the GSSG 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. Note: Since one GSSG molecule can be reduced to two GSH molecules after the reaction, the concentration of GSSG needs to be multiplied by 2 when converted to the concentration of GSH. For example, if the endogenous GSH in the sample is completely removed, the concentration of GSSG is 5μM, which is equivalent to the concentration of GSH being 10μM.
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. According to the ΔA412/min of the sample, the total glutathione or GSSG content in the sample during the determination can be calculated by comparing with the standard curve.
3. At the same time, the total glutathione or GSSG content per milligram of tissue or cell can be calculated based on the dilution multiple of the sample and the initial sample usage. For cell samples, the protein content of the cell sample can also be calculated based on the initial number of cells used, and then a certain number of cells are taken for lysis and the protein concentration is measured, and finally the total glutathione or GSSG content per milligram of protein is calculated.

4. The GSH content can be calculated based on the total glutathione content and GSSG content obtained by the determination. The calculation formula is: GSH = Total Glutathione-GSSG × 2 (Note: Total Glutathione is the GSSG concentration calculated by the standard curve multiplied by 2, and the GSSG obtained after removing GSH should also be multiplied by 2, because 1 GSSG molecule can be reduced to 2 GSH molecules after the reaction). For example, the concentration of total glutathione (Total Glutathione) determined by this kit is 15μM (that is, the GSSG concentration calculated by the standard curve when determining the total glutathione is 7.5μM, multiplied by 2 to get the total glutathione concentration), and the concentration of GSSG determined is 1.2μM (that is, the GSSG concentration calculated by the standard curve when determining the GSSG content alone is 1.2μM), then the concentration of GSH in the sample is 15-1.2×2=12.6μM.

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. Reagents such as NADPH are not very stable, so please strictly follow the subsequent instructions to prevent inactivation.
4. Protein Removal Reagent M Solution must be freshly prepared and used on the same day. GSH Scavenging Reagent must also be freshly diluted before use.
5. be dissolved by vigorous vortexing and appropriate heating (not exceeding 37 º C).
6. DMSO will solidify at low temperatures such as 4ºC or ice bath. It can be incubated in a 20-25 º C water bath for a while until it is completely melted before use.
7. 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.