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

Reduced Glutathione (GSH) Assay Kit (DTNB Method)

Catalog No.: BC00025

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

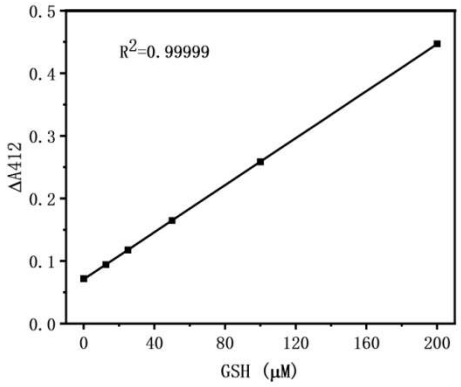
Product Name	Reduced Glutathione (GSH) Assay Kit (DTNB Method)
Detection Method	Colorimetric
Sample Type	Tissues and cells
Assay Type	Quantitative
Detection Instrument	Microplate reader (412 nm)

Product Introduction

Glutathione is a small peptide composed of three amino acid residues. Its full name is glutamyl-cysteinyl-glycine, and its English name is glutamyl-cysteinylglycine, or glutathione for short. 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 through thiol groups, it is often abbreviated as G-S-S-G 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 reduced glutathione in samples containing protein. The actual measurement effect of the standard product is shown in the figure below :



Principle

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. Therefore, the amount of reduced glutathione can be calculated by measuring A412. The specific reaction principle of this kit is as follows:

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

Components

Serial number	Components	Size(100T)	Storage
Reagent 1	Reduced Glutathione Assay Buffer	60ml	-20℃
Reagent 2	Reduced Glutathione (GSH)	4.5mg	-20℃, prepare into solution, store at -20ºC after aliquoting.
Reagent 3	DTNB	4.5mg	-20℃, prepare into solution, store at -20ºC after aliquoting.
Reagent 4	Protein Removal Reagent S	0.4g	-20℃, prepare solution and store at 2-8 ºC.
Reagent 5	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 10mg 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 reduced 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 the assay instead of frozen cells. Wash the cells once with PBS, collect the 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 10 mg 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 on ice for 5 minutes. Centrifuge at 10,000 x g for 10 minutes at 4°C. Take the supernatant for the determination of reduced 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 the determination of reduced 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 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 8ml of Milli-Q grade water to 0.4g of Protein Removal Reagent S provided in this kit to prepare 8ml of 5% aqueous solution. Store at 4ºC.

4. Preparation of reduced glutathione detection working solution: According to the number of samples to be tested, refer to the table below to prepare an appropriate amount of reduced glutathione detection working solution. The reduced glutathione detection working solution is obtained by mixing the two reagents in the table in proportion.

	1 sample	10 samples	20 samples
DTNB Stock Solution	6.6 μl	66 μl	132 μl
Reduced Glutathione Assay Buffer	150 μl	1.5 ml	3 ml

5. Preparation of standard: Dilute 10mM GSH stock solution with water to 50μM GSH solution. Then dilute it to 25 , 12.5 , 6.25 , 3.125μM GSH solution in half dilution method. Take 50, 25 , 12.5 , 6.25 , 3.125 , 0 (blank well) μM GSH solution as seven points to make a standard curve.

Operation process

1. Use a 96-well plate, add 150μl of reduced glutathione detection working solution to each well, then add 50 μl of sample or standard, mix well, and incubate at 25ºC or room temperature for 5 minutes.
2. A412 using an appropriate microplate reader or microvolume UV spectrophotometer .

Result calculation

Using the A412 of the standard substance to obtain a linear fitting equation, and the content of reduced glutathione is calculated by substituting the A412 of the sample into the equation. At the same time, the content of reduced glutathione per milligram of tissue or cell can be calculated according to the dilution multiple of the sample and the initial sample usage. For cell samples, the protein concentration can also be determined after a certain number of cells are lysed based on the initial number of cells used, thereby calculating the protein amount of the cell sample, and finally calculating the content of reduced glutathione 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. 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.
3. 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.