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

Glutathione Reductase (GR) Activity Assay Kit (DTNB Method)

Catalog No.: BC00024

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

Website: www.enkilife.com

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	Glutathione Reductase (GR) Activity Assay Kit (DTNB Method)
Detection Method	Colorimetric
Sample Type	Tissue, Cells
Assay Type	Enzyme activity
Detection Instrument	Microplate reader (412 nm)

Product Introduction

Glutathione reductase is distributed in many tissues and can maintain sufficient reduced glutathione (GSH) levels in cells.

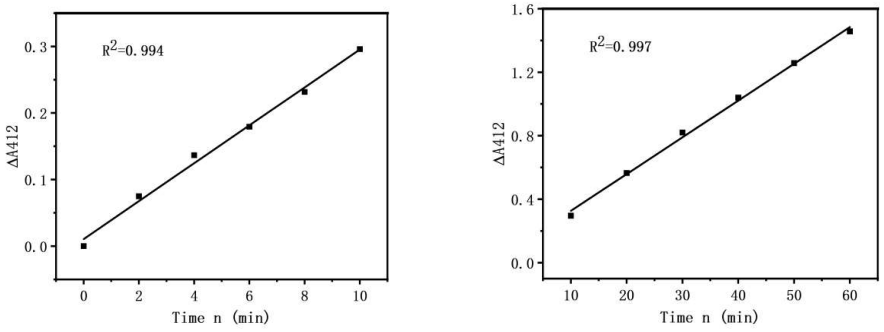
Principle

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, when GSSG/GSH is excessive and GR is relatively insufficient, the glutathione reductase in the reaction system becomes the rate-limiting factor of the entire reaction system. At this time, the amount of yellow TNB generated is linearly positively correlated with the activity of glutathione reductase. Therefore, the activity level of glutathione reductase can be calculated by measuring A412. The specific reaction principle of this kit is as follows:

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

$$\mathrm{2GSH + DTNB \rightarrow GSSG + 2TNB}$$

The detection effect of this kit on 37μg liver sample is shown in the figure below



Components

Serial number	Components	Size(100T)	Storage
Reagent 1	Glutathione reductase assay	50ml	-20℃
Reagent 2	Sample diluent	50ml	-20℃
Reagent 3	NADPH	5mg	-20℃, after NADPH is dissolved, it should be appropriately divided and stored at -70° C. It can be stored at 4° C for one day. After being stored at -20° C for one week, NADPH will degrade by more than 10%.
Reagent 4	Oxidized glutathione (GSSG)	14.2mg	-20℃, GSSG is prepared into solution and stored at -20° C after packaging.
Reagent 5	DTNB	4.5mg	-20℃, DTNB is prepared into solution and stored at -20° C after aliquoting.
Reagent 6	DMSO	1.5ml	-20℃
Consumable 1	Microplate(96 wells)	1 plate	RT
Consumable 2	Plate Sealer	2 pieces	RT

Storage

Unopened kits can be stored at -20℃ for 12 months.

Experimental Preparation

- Sample processing

1. Preparation of cell samples: For adherent cells, trypsin digestion should be avoided because they will be used for enzyme activity determination later. Cells can be washed once with PBS, HBSS or saline. For suspended cells, cells can be collected by centrifugation and washed once with PBS, HBSS or saline. Subsequently, EnkiLife's Western and IP Cell Lysis Buffer **(RC0009)** can be used to lyse the cell samples according

to the corresponding instructions. Lyse by adding 100-200μl of lysis buffer directly to per 1 million cells. For adherent cells, a cell spatula or cell scraper can be used to assist in collecting cell samples. If the lysis effect is not good, the cell samples in the lysis buffer can be homogenized with a glass homogenizer at 4°C or in an ice bath. Then centrifuge at 4°C, 12,000×g for 10 minutes. The supernatant is used for enzyme activity determination.

2. Preparation of tissue samples: The animals are perfused with saline containing 0.16mg/ml heparin (0.9% NaCl containing 0.16mg/ml heparin) to remove blood and obtain tissue samples. Add 200μl of sample Western and IP Cell Lysis Buffer **(RC0009)** or other appropriate homogenate to every 20 mg of tissue, and homogenize with a tissue grinder or glass homogenizer at 4°C or in an ice bath.Centrifuge at 12,000 x g for 10 minutes at 4°C. Take the supernatant for enzyme activity determination.

3. Preparation of red blood cell lysis buffer: Collect blood in an anticoagulant tube and mix by inversion. Take at least 500μl of whole blood and centrifuge at 2500 x g for 5 minutes at 4°C. Discard the supernatant, resuspend the pellet with ice-cold Western and IP Cell Lysis Buffer **(RC0009)** or other appropriate homogenate in an amount of about 10 times the volume of the red blood cell pellet, centrifuge again as before, and discard the supernatant. Add ice-cold Milli-Q grade pure water in an amount of about 4 times the volume of the red blood cell pellet to lyse the red blood cells. Centrifuge at 12,000 x g for 5 minutes and take the supernatant.

4. The above samples can be tested for protein concentration using BCA Protein Assay Kit (BCA Method)**(BC00006)** produced by EnkiLife. Usually, a sample containing 1-100μl of protein can be used for glutathione reductase detection.

Note: For samples with higher GR activity, samples containing 1-10μl of protein may be sufficient for detection, while for samples with lower GR activity, 10-100μl of protein may be required. If the activity of glutathione reductase in the sample is found to be too high, it can be diluted with sample diluent. If the activity of glutathione reductase in the sample is too low, the amount of protein should be increased appropriately. If the prepared sample is to be tested on the same day, it can be stored in an ice bath, and if it is to be tested later, it can be frozen at -70°C.

- Preparation of the assay kit

1. Preparation of 6mM NADPH solution.

Add 1ml of Milli-Q grade pure water to the 5mg NADPH provided in this kit, dissolve and mix to obtain a 6mM NADPH solution. Except for the portion to be used immediately, the remaining NADPH solution should be appropriately aliquoted and stored at -70°C.

2. Preparation of GSSG solution.

Add 10ml of Milli-Q grade pure water to the 14.2mg GSSG provided in this kit, dissolve and mix. Except for the portion to be used immediately, the remaining GSSG solution should be appropriately aliquoted and stored at -20° C.

3. Preparation of DTNB solution.

Add 1.5ml of DMSO provided in this kit to the 4.5mg DTNB provided in this kit, dissolve and mix. Except for the portion to be used immediately, the remaining DTNB solution should be appropriately aliquoted and stored at -20° C away from light.

4. All reagents must be incubated to 25° C in a water bath or on a PCR instrument before use.

Operation process

1. Refer to the table below, use a 96-well plate, add each solution in turn and mix well.

	Blank	**Sample**
GSSG Solution	100μl	100μl
Glutathione Reductase Assay Buffer	90μl	70-90μl
Sample	0μl	0-20μl
6mM NADPH Solution	10μl	10μl
Total Volume	200μl	200μl

2. Add 6.6μl of DTNB solution to each well and mix well.

3. Immediately use an appropriate microplate reader or micro-volume UV spectrophotometer to measure A412, and record it as the 0-minute reading, i.e. A412 (Time 0). If the instrument can set the temperature, set it to 25° C. Otherwise, adjust the room temperature to 25° C through the air conditioner, and start measuring A412 after the instrument is expected to reach 25° C.

4. Measure A412 every 2 minutes, i.e. A412 (Time n), and record it continuously for at least 10 minutes to obtain data at 5 points. Or if the instrument has the corresponding function, let the instrument measure continuously for 10 minutes or automatically measure A412 every 2 minutes. If the absorbance of the sample is relatively low, the incubation time can be extended. The absorbance of the sample will increase linearly with the extension of time within a certain range. At this time, consider measuring the absorbance every 10 minutes.

Note:

ΔA340(blank)=A340(blank)(Time 0) - A340(blank)(Time n)

ΔA340(sample)=A340(sample)(Time 0) - A340(sample)(Time n)

ΔA340=ΔA340(sample) - A340(blank)

ΔA340/min=ΔA340/n

Result calculation

1. Definition of glutathione reductase activity unit: 1 unit of enzyme activity (1 unit) can reduce 1μmol of GSSG in 1 minute at 25°C and pH 7.5. 1 U=1000 mU.
2. When calculating, the A412/min of each sample needs to be deducted from the A412/min measured by the corresponding sample background control. The measured △A412/min should preferably be controlled within the range of 0.005-0.1. If the measured △A412/min value is too large, the sample can be appropriately diluted or the sample dosage can be reduced. If the △A412/min value is too small, the sample should be concentrated as much as possible and the sample dosage should be appropriately increased when processing the sample.

Note:

ΔA412 (blank)=A412 (blank, Time n)-A412 (blank, Time 0);

ΔA412 (sample)=A412 (sample, Time n)-A412 (sample, Time 0);

ΔA412=ΔA412 (sample)-ΔA412 (blank);

ΔA412/min=ΔA412/n

3. For glutathione reductase: 1mU/ml＝1nmol TNB/min/ml＝(ΔA412/min)/($\varepsilon^{\mu M}$×L(cm))

[Glutathione reductase activity in the test system]= (ΔA412/min)/($\varepsilon^{\mu M}$×L(cm))=[(ΔA412 (sample)-ΔA412 (blank))/min]/ ($\varepsilon^{\mu M}$×L(cm))

[Glutathione reductase activity in the sample]＝[Glutathione reductase activity consumed in the test system]×[dilution factor]/[protein concentration in the sample] = [(ΔA412/min)/($\varepsilon^{\mu M}$×L(cm))]×[dil×(V(ml)/Vsample(ml))]/[protein concentration in the sample]

Note: The unit of [glutathione reductase activity in the detection system] is mU/ml, and the unit of [protein concentration in the sample] is mg/ml, so the final unit of [glutathione reductase activity in the sample] is: U/mg protein or mU/mg protein;

$\varepsilon^{\mu M}$ is the molar extinction coefficient: the molar extinction coefficient of TNB in A412 is

0.01415μM^{-1}cm^{-1};
L(cm) is the path length when measuring absorbance: the height of 200μl sample in a general 96-well is about 0.552cm. If different reaction wells are used, please note that it is modified to the height of the solution in the well;
dil is the dilution multiple of the sample;
V(ml) is the reaction system, which is 0.2ml in this reaction system;
V_{sample}(ml) is the volume of the sample in the reaction system, expressed in ml.
4. Calculation example: The protein concentration of the sample is determined to be 5mg/ml. After diluting 10 times with sample diluent, take 10μl of the diluted sample and refer to Table 1 for measurement. If ΔA412/min(sample)＝0.048, ΔA412/min(blank)＝0.006, then:
[Glutathione reductase activity in the detection system]= (0.048-0.006)/(0.01415×0.552) =5.38mU/ml
[Glutathione reductase activity in the sample]= 5.38mU/ml×10×0.2/0.01/(5mg/ml)= 0.2152U/mg(protein)

Notes

1. This kit involves redox reactions, so all oxidants or reductants will interfere with the determination of this kit. If the reducing agent in the sample cannot be avoided, such as DTT, mercaptoethanol, etc., the total concentration of these reducing agents should be at least less than 0.1mM. 0.15mM DTT can inhibit 40% of the enzyme activity. In addition, sodium sulfate, ammonium sulfate and ferrocyanide will interfere with the determination of this kit. Please try to avoid.
2. The sample can be measured immediately or frozen at -70°C for later determination.
3. The temperature during the reaction must be strictly controlled to 25°C, otherwise it will cause more errors.
4. NADPH is not very stable, so follow the subsequent instructions strictly to prevent inactivation.
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.