CheKine™ Micro Glutathione Oxidized (GSSG) Assay Kit

Cat #: KTB1610 Size: 96 T/96 S

	Micro Glutathione Oxidized (GSSG) Assay Kit		
REF	**Cat #:** KTB1610	LOT	**Lot #:** Refer to product label
	Detection range: 1-20 μM		**Sensitivity:** 1 μM
	Applicable samples: Serum, Plasma, Animal/Plant Tissues, Blood cells, Cells, Bacteria		
	Storage: Stored at -20℃ for 12 months, protected from light		

Assay Principle

Glutathione has two forms: reduced form (GSH) and oxidized form (GSSG). GSSG also known as Dithioglutathione, is formed by the oxidation of two molecules of glutathione. GSSG will be reduced to GSH by glutathione reductase (GR), so most of it exists in the reduced form in the body. Determination of intracellular GSH and GSSG content and GSH/GSSG ratio can well reflect the redox state of cells, and is also one of the main indicators of glutathione redox cycle. CheKine™ Micro Glutathione Oxidized (GSSG) Assay Kit provides a simple, convenient and rapid method for the detection of GSSG content, which is suitable for serum, plasma, animal and plant tissues, blood cells, cells, bacteria, etc. sample. The principle is that reduced Glutathione can react with 5,5-dithiobis (2-nitrobenzoic acid) (DTNB) to generate 2-nitro-5-mercaptobenzoic acid, which has a maximum light at a wavelength of 412 nm. Absorption, inhibit the original reduced glutathione in the sample by 2-vinylpyridine, and then use GR to reduce GSSG to GSH, thereby determining the content of oxidized glutathione.

Materials Supplied and Storage Conditions

Kit components	Size (96 T)	Storage conditions
Extraction Buffer	70 mL×2	4℃
Inhibitor	210 μL	-20℃, protected from light
Assay Buffer	20 mL	4℃
GR	14 μL	4℃, protected from light
GR Cofactor	Powder×2 vials	-20℃, protected from light
Chromogen	Powder×2 vials	4℃, protected from light
Standard	Powder×1 vial	4℃, protected from light

Note: Before formal testing, it is recommended to select 2-3 samples with large expected differences for pre-experiment.

Materials Required but Not Supplied

- Microplate reader or visible spectrophotometer capable of measuring absorbance at 412 nm

- 96-well plate or microglass cuvette, precision pipettes, disposable pipette tips
- Refrigerated centrifuge, water bath
- Deionized water, PBS
- Homogenizer (for tissue samples)

Reagent Preparation

Extracting Buffer: Ready to use as supplied. Equilibrate to room temperature before use. Store at 4℃.

Inhibitor: Ready to use as supplied. Equilibrate to room temperature before use. Store at -20℃, protect form light.

Note: The Inhibitor is toxic and has a pungent odor, so it is recommended to experiment in a fume hood.

Assay Buffer: Ready to use as supplied. Equilibrate to room temperature before use. Store at 4℃.

Diluted GR solution: Based on samples number, the ratio of adding 1 μL GR into 20 μL deionized water is freshly prepared. Mix well and protected from light.

Diluted GR Cofactor Solution: Add 1.5 mL of deionized water into each GR Cofactor before use. Store at -20℃ for 1 month, protected from light.

Diluted Chromogen Solution: Add 1.5 mL of deionized water into each Chromogen before use. Store at 4℃ for 1 month, protected from light.

Standard preparation:

Diluted Extraction Buffer: Make a 1:10 dilution of the Extraction Buffer solution with deionized water in a clean plastic tube by diluting 250 μL Extraction Buffer into 2,250 μL deionized water to dilute the standard.

20 mM GSSG Standard: Take 1 vial Standard and dissolve with 1 mL Diluted Extraction Buffer.

20 μM GSSG Standard: Prepare 2 mM GSSG Standard by diluting 100 μL 20 mM GSSG Standard into 900 μL Diluted Extraction Buffer. Prepare 20 μM GSSG Standard by diluting 10 μL 2 mM GSSG Standard into 990 μL Diluted Extraction Buffer.Using 20 μM GSSG Standard prepare standard curve as described:

Num.	20 μM Standard (μL)	Diluted Extraction Buffer (μL)	Concentration (μM)
Std.1	100	0	20
Std.2	80	20	16
Std.3	60	40	12
Std.4	40	60	8
Std.5	20	80	4
Std.6	10	90	2
Std.7	5	95	1

Notes: Always prepare fresh standards per use; Diluted Standard Solution is unstable and must be used within 4 h.

Sample Preparation

Note: Fresh samples are recommended, If not assayed immediately, samples can be stored at -80℃ for 10 days.

1. Animal and Plant Tissues: Please try to use fresh tissue samples for determination. Weigh 0.1 g tissues, add 1 mL Extraction Buffer and homogenize on ice (the homogenizer is pre-cooled in advance). Centrifuge at 8,000 g for 10 min at 4℃. Use supernatant for assay, and place it on ice to be tested.
2. Plasma or Serum: Please try to use fresh serum (plasma) for determination. Collect plasma or serum using an anticoagulant. Centrifuge at 600 g for 10 min at 4℃. Collect supernatant within 30 min and add equal volume of Extraction Buffer. Centrifuge at 8,000 g for 10 min at 4℃. Use supernatant for assay, and place it on ice to be tested.
3. Blood Cells: Please try to use fresh blood cells for determination. Collect blood using an anticoagulant. Centrifuge at 600 g for 10 min at 4℃. Discard the upper plasma, then wash the pellet with triple volume of cold PBS 2-3 times (use PBS resuspend blood

cells, centrifuge at 600 g for 10 min at 4℃). Add equal volume of Extraction Buffer, then mix and stand at 4℃ for 10 min. Centrifuge at 8,000 g for 10 min at 4℃. Use supernatant for assay, and place it on ice to be tested.

4. Cells or Bacteria: Please try to use fresh cells (bacteria) for determination, rather than using frozen cells (bacteria) for determination. Collect 5×10^6 cells or bacteria for each assay. Wash cells or bacteria with cold PBS twice (resuspend with PBS, centrifuge at 600 g for 10 min at 4℃). Resuspend in triple volume of cells or bacteria pellet Extraction Buffer, then ultrasonic break in ice bath 5 min (power 20% or 200 W, ultrasonic 3 s, interval 7 s, repeat 30 times) . Centrifuge at 8,000 g for 10 min at 4℃. Use supernatant for assay, and place it on ice to be tested. Cells can also be extracted by freeze-thaw method, and bacteria can not be used. The resuspended cells could rapidly freeze and thaw 2-3 times (frozen in liquid nitrogen, dissolved in 37℃ water bath). Centrifuge at 8,000 g for 10 min at 4℃. Use supernatant for assay, and place it on ice to be tested.

Note: Because the Extraction Buffer contains a protein precipitator, the supernatant cannot be used for protein concentration determination. If the protein content needs to be determined, the same sample needs to be taken and the Extraction Buffer replaced with deionized water for extraction preparation. It will be better to quantify the total protein with Protein Quantification Kit (BCA Assay), Cat #: KTD3001, if the content is calculated by protein concentration.

Assay Procedure

1. Preheat the microplate reader or visible spectrophotometer for more than 30 min, and adjust the wavelength to 412 nm, visible spectrophotometer was returned to zero with deionized water.

2. Add the following reagents to the 1.5 mL EP tube:

Reagent	Blank Well (μL)	Standard Well (μL)	Test Well (μL)
Sample	0	0	3
Deionized Water	30	0	27
Different Concentrations Standard	0	30	0
Inhibitor	1.5	1.5	1.5
Mix well, incubate for 30 min at 37℃, that is, the mixture. **Note: 1. This step must be mixed in 1.5mL EP tube, which can not be directly added to the 96-well plate, which will corrode the well plate. 2. The Inhibitor is toxic and has a pungent odor, so it is recommended to experiment in a fume hood.**			
Add the following reagents to the 96-well plate or microglass cuvette:			
Mixture	21	21	21
Assay Buffer	140	140	140
Diluted GR Solution	2	2	2
Diluted GR Cofactor Solution	20	20	20
Diluted Chromogen Solution	20	20	20

3. Mix well, immediately detect optical density at 412 nm as A_1. Incubate for 10 min at 37°C in the dark. Measure optical density of 10 min at 412 nm again as A_2, $\Delta A= A_2-A_1$.

Note: 1. If the ΔA_{Test} value of Samples are higher than the $\Delta A_{Standard}$ value of the 20 μM Standard, dilute sample with deionized water and repeat this assay and increase the sample size if the ΔA_{Test} value is below 0.005. 3. The reaction is relatively rapid, immediately after mixing began to read the A value, do not detect too many samples at a time.

Data Analysis

The measured absorbance values of standard well and test well should minus the absorbance of blank well, that is, $\Delta\Delta A_{Standard}=\Delta A_{Standard}-\Delta A_{Blank}$, $\Delta\Delta A_{Test}=\Delta A_{Test}-\Delta A_{Blank}$.

1. Drawing the standard curve:

With the concentration of the Standard Solution as the y-axis and the $\Delta\Delta A_{Standard}$ as the x-axis, draw the standard curve. Substitute the $\Delta\Delta A_{Test}$ into the equation to obtain the y value (μM).

2. Calculate the content of GSSG in sample

(1) By sample fresh weight

GSSG (nmol/g)=$y \times V_{Standard} \div V_{Sample} \times V_{Extraction} \div W \times n$=**$10 \times y \div W \times n$**

(2) Calculated by protein concentration

GSSG (nmol/mg prot)=$y \times V_{Standard} \div V_{Sample} \div Cpr \times n$=**$10 \times y \div Cpr \times n$**

(3) Calculated by cells or bacteria number

GSSG (nmol/10^4)=$y \times V_{Standard} \div V_{Sample} \times V_{Extraction} \div 500 \times n$=**$0.02 \times y \times V_{Extraction} \times n$**

(4) Calculated by liquid volume

GSSG (nmol/mL)=$y \times V_{Standard} \div V_{Sample} \times 2 \times n$=**$20 \times y \times n$**

Where: nmol: 1 μM=1 nmol/mL; $V_{Standard}$: Standard volume added, 30 μL; V_{Sample}: Sample volume added, 3 μL; $V_{Extraction}$: Extraction Buffer volume added, 1 mL (The cells or bacteria shall be subject to actual volume); W: sample weight, g; n: Dilution factor; Cpr: Supernatant protein concentration, mg/mL; 500: Total number of cells or bacteria, 5×10^6; 2: Double the dilution during liquid extraction.

Typical data

Typical standard curve

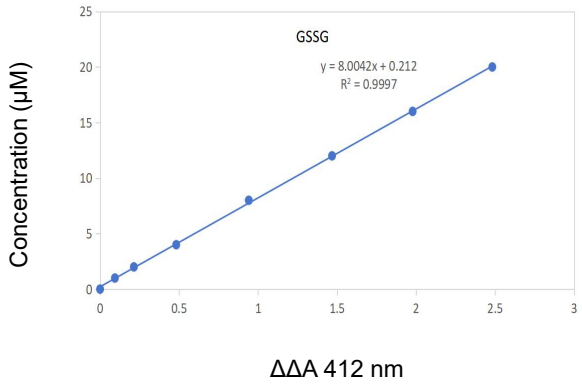

Figure 1. Standard Curve for GSSG.

Recommended Products

Catalog No.	Product Name
KTB1600	CheKine™ Micro Reduced Glutathione (GSH) Assay Kit
KTB1620	CheKine™ Micro Glutathione Reductases (GR) Activity Assay Kit
KTB1630	CheKine™ Micro Glutathione S-Transferase (GST) Assay Kit
KTB1640	CheKine™ Micro Glutathione Peroxidase (GSH-Px) Activity Assay Kit
KTB1650	CheKine™ Micro Thioredoxin Reductase (TrxR) Assay Kit

Disclaimer

The reagent is only used in the field of scientific research, not suitable for clinical diagnosis or other purposes. For your safety and health, please wear a lab coat and disposable gloves.