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CheKine™ Micro Total glutathione（T-GSH）/Oxidized glutathione（GSSG） Assay Kit

Cat #: KTB1671 Size: 48 T/ 24 S 96 T/48 S

	CheKine™ Micro Total glutathione （T-GSH）/Oxidized glutathione（GSSG） Assay Kit		
REF	**Cat #:** KTB1671	LOT	**Lot #:** Refer to product label
	Detection range: 0.78-50 μM (T-GSH); 0.78-25 μM (GSSG)		**Sensitivity:** 0.78 μM
	Applicable samples: Serum, Plasma, Animal/Plant Tissues, Whole blood, Blood cells, Cells, Bacteria		
	Storage: Stored at -20℃ for 12 months, protected from light		

Assay Principle

The total 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), therefore most of glutathione exist in the reduced form. 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 Total glutathione （T-GSH）/Oxidized glutathione（GSSG） Assay Kit provides a simple, convenient and rapid method for the detection of T-GSH and GSSG content, which is suitable for serum, plasma, animal and plant tissues, whole blood, blood cells, cells, bacteria, etc. sample. The principle is that GSSG was reduced to GSH. The reduced Glutathione can react with 5,5-dithiobis (2-nitrobenzoic acid) (DTNB) to generate 2-nitro-5-mercaptobenzoic acid, which has a maximum absorption at wavelength of 412 nm, thereby determining the content of total GSH by absorbance changes. If the original reduced glutathione in the sample was inhibited by 2-vinylpyridine, and then use GR to reduce GSSG to GSH, thereby determining the content of oxidized glutathione. The reduced glutathione can be calculated by substract GSSG from total GSH.

Materials Supplied and Storage Conditions

Kit components	Size		Storage conditions
	48 T	96 T	
Extraction Buffer	70 mL	70 mL×2	4℃
Inhibitor	105 μL	210 μL	-20℃, protected from light
Assay Buffer	12.5 mL	25 mL	4℃
GR	7 μL	14 μL	4℃, protected from light
GR Cofactor	Powder×1 vial	Powder×1 vial	-20℃, protected from light
Chromogen	1.5 mL	3 mL	4℃, protected from light
GSH Standard	Powder×2 vials	Powder×2 vials	4℃, protected from light
GSSG Standard	Powder×1 vial	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 (405 nm as alternative)
- 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 solution: Used for GSSG test, based on sample numbers, adding 1.5 μL inhibitor into 160 μL Assay Buffer per well. 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 GR and deionized water is 1:20. Mix well and protected from light.

Diluted GR Cofactor Solution: For 48 T, add 1.5 mL of deionized water into GR Cofactor before use; For 96 T, add 3.0 mL of deionized water into GR Cofactor before use. Aliquoted and store at -20℃ for 1 month, protected from light.

Enzyme working solution: Prepared before use. Based on samples number, mix 2 μL Diluted GR solution and 20 μL Diluted GR Cofactor Solution well for each well.

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

Standard preparation:

10 mM GSSG or GSH Standard: Take 1 vial Standard and dissolve with 1 mL deionized water.

100 μM GSSG or GSH Standard: Prepare 100 μM GSSG or GSH standard by diluting 10 μL 10 mM GSSG or GSH standard into 990 μL Extraction Buffer. Using 100 μM GSSG or GSH standard prepare standard curve as described: For T-GSH detection, 0 - 50 μM was used. While 0 - 25 μM was used for GSSG detection.

Num.	Standard (μL)	Extraction Buffer (μL)	Concentration (μM)
Std.1	100 μM 100 μL	100	50
Std.2	50 μM 100 μL	100	25
Std.3	25 μM 100 μL	100	12.5
Std.4	12.5 μM 100 μL	100	6.25
Std.5	6.25 μM 100 μL	100	3.125
Std.6	3.125 μM 100 μL	100	1.5625
Std.7	1.5625 μM 100 μL	100	0.78125
Blank	0	100	0

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 30 days. Since the supernatant was deproteinated and cannot be directly used for protein concentration measurement. If protein content determination is required, a separate aliquot of the same sample must be processed by replacing the Extraction Buffer with deionized water. This kit involves redox reactions. Reagents with reducing or oxidizing properties, such as DTT, TCEP, β-mercaptoethanol, iron ions, cysteine, etc., may interfere with the reaction. Additionally, Tris can also affect the reaction system. Therefore, the use of reagents containing these substances should be avoided during sample

preparation. The use of zirconium oxide grinding beads for sample grinding is recommended. Ensure the probe has not been rusted before performing ultrasonic disruption.

1. Animal and Plant Tissues: Please try to use fresh tissue samples for determination. Weigh 0.1 g tissues, add 1 mL ice-cold 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. Mix plasma or serum with Extraction Buffer at a ratio of 1:3. Keep at room temperature for 5 min. Centrifuge at 8,000 g for 10 min at 4℃. Use supernatant for assay, and place it on ice to be tested.

3. 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 cells or bacteria pellet using 0.2 mL 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, 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.

4. whole blood: Please try to use fresh whole blood samples for determination. Mix whole blood with Extraction Buffer at a ratio of 1:3. and homogenize on ice (the homogenizer is pre-cooled in advance). Centrifuge at 8,000 g for 10 min at 4℃. To avoid surface pellicle interfere, take the supernatant and centrifuge at 8,000 g for 1 min at 4℃.Use supernatant for assay, and place it on ice to be tested.

5. Blood cells: Please try to use fresh blood cells for determination. Collect blood using an anticoagulant. Centrifuge at 1000 g for 10 min at 4℃. Discard the upper plasma and white blood cells. Then wash the pellet twice with five times volume of cold PBS, centrifuge at 1000 g for 10 min at 4℃. then break blood cells by grinding or ultrasonic method as described above. Centrifuge at 8,000 g for 10 min at 4℃. Use supernatant for assay, and place it on ice to be tested.

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. For GSSG Assay: add the following reagents to the 96-well plate

Reagent	Blank Well (μL)	Standard Well (μL)	Test Well (μL)
sample	0	0	20
GSSG standard	0	20	0
Extraction Buffer	20	0	0
Inhibitor solution	160	160	160
Mix well and keep at 37℃ for 30 min			
Enzyme working solution	22	22	22
Chromogen	20	20	20

3. Mix well, immediately detect optical density at 412 nm as A_1. Incubate for 15 min at room temperature in the dark. Measure optical density of 15 min at 412 nm again as A_2, $\Delta A= A_2-A_1$.

4. For T-GSH Assay: add the following reagents to the 96-well plate

Reagent	Blank Well (μL)	Standard Well (μL)	Test Well (μL)
sample	0	0	20
GSH standard	0	20	0
Extraction Buffer	20	0	0
Assay Buffer	160	160	160
Enzyme working solution	22	22	22
Chromogen	20	20	20

5. Mix well, immediately detect optical density at 412 nm as A_1. Incubate for 5 min at room temperature in the dark. Measure optical density of 5 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 highest concentration standard, dilute sample with Extraction Buffer. Increase the sample size or reduce Extraction Buffer if the ΔA_{Test} value is below the $\Delta A_{Standard}$ value of the 0.78 μM Standard. 2. The reaction is relatively rapid, immediately read the A value after mixing, do not detect too many samples at a time. 3. Adding of Chromogen into Standard well from low concentration to high concentration was recommended.

Data Analysis

The measured absorbance values of standard well and test well should minus the absorbance of blank well, that is $\Delta A = A_2 - A_1$, $\Delta\Delta A_{Standard} = \Delta A_{Standard} - \Delta A_{Blank}$, $\Delta\Delta A_{Test} = \Delta A_{Test} - \Delta A_{Blank}$.

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)=$y \times V_{sample} \div (W \times V_{Sample} \div V_{Extraction}) \times n = \mathbf{y \div W \times n}$

(2) Calculated by protein concentration

GSSG (nmol/mg prot)=$y \times V_{sample} \div (V_{Sample} \times Cpr) \times n = \mathbf{y \div Cpr \times n}$

(3) Calculated by cells or bacteria number

GSSG (nmol/10^4)=$y \times V_{sample} \div (N \times V_{Sample} \div V_{Extraction}) \times n = \mathbf{y \div N \times V_{Extraction} \times n}$

(4) Calculated by liquid volume

GSSG (nmol/mL)$\mathbf{= y \times n}$

3. Calculate the content of T-GSH in sample

(1) By sample fresh weight

T-GSH (nmol/g)=$y \times V_{sample} \div (W \times V_{Sample} \div V_{Extraction}) \times n = \mathbf{y \div W \times n}$

(2) Calculated by protein concentration

T-GSH (nmol/mg prot)=$y \times V_{sample} \div (V_{Sample} \times Cpr) \times n = \mathbf{y \div Cpr \times n}$

(3) Calculated by cells or bacteria number

T-GSH (nmol/10^4)=$y \times V_{sample} \div (N \times V_{Sample} \div V_{Extraction}) \times n = \mathbf{y \div N \times V_{Extraction} \times n}$

(4) Calculated by liquid volume

T-GSH (nmol/mL)$\mathbf{= y \times n}$

4. Calculate the content of GSH in sample

GSH=T-GSH - GSSG*2

Where: nmol：1 μM=1 nmol/mL；V_{Sample}：Sample volume added，20 μL；$V_{Extraction}$：Extraction Buffer volume added，1 mL（For other samples, the volume can be adjusted according to actual conditions）；W：sample weight，g；n：Dilution factor；Cpr：

Supernatant protein concentration, mg/mL; N: number of cells or bacteria, take 10^4 as one unit (For example, cell number is 5×10^6, N=500).

Typical data

Typical standard curve: y=83.845x-0.1555, R^2=0.9968; y=122.49x-0.8265, R^2=0.9964.

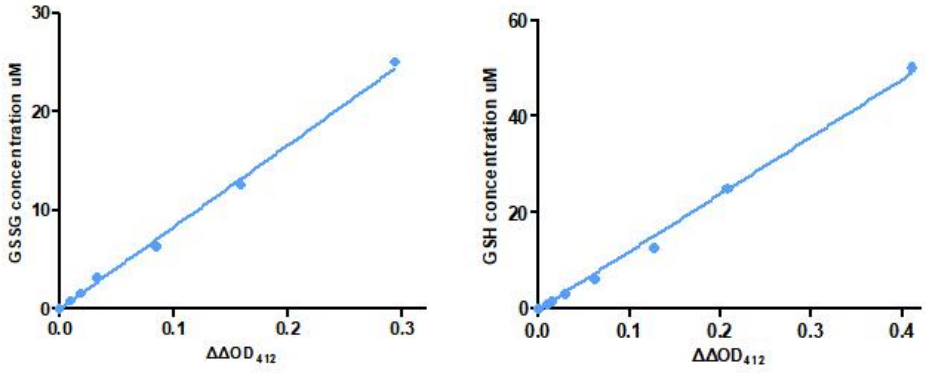

Figure 1. Standard Curve for GSSG and GSH.

Recommended Products

Catalog No.	Product Name
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.