CheKine™ Micro Total Glutathione (T-GSH) Assay Kit

Cat #: KTB1670 Size: 48 T/96 T

	Micro Total Glutathione (T-GSH) Assay Kit		
REF	**Cat #:** KTB1670	LOT	**Lot #:** Refer to product label
	Detection range: 0.04-4 ug/mL		**Sensitivity:** 0.04 ug/mL
	Applicable samples: Animal and Plant Tissues, Bacteria, Cells, Serum		
	Storage: Stored at -20℃ for 12 months, protected from light		

Assay Principle

Glutathione is a Tripeptide of Glycine, Glutamic acid and Cysteine. In the red blood cell, reduced Glutathione (GSH) is the key to maintain hemoglobin in reduced state and protect cells from oxidative damage. GSH is the most important antioxidant sulfhydryl compound in cells, which plays an important role in oxidation resistance, protein sulfhydryl protection and amino acid transport across membrane. The ratio between reduced and oxidized Glutathione (GSH/GSSG) is the main indicator of the cell's redox state. Therefore, measuring the content of GSH and GSSG and the ratio of GSH/GSSG in cells can reflect the redox state of cells. CheKine™ Micro Total Glutathione (T-GSH) Assay Kit can detect samples of animal and plant tissues, bacteria, cells, serum, etc. Glutathione reductase (glutathione reductase, GR) is used to reduce GSSG to GSH. GSH can react with 5,5'-Dithiobis-(2-Nitrobenzoic Acid) (5,5'-Dithiobis-2-Nitrobenzoic Acid, DTNB) to form 2-Nitro-5-Diol Benzoic Acid. This substance is yellow and has a maximum light absorption at a wavelength of 412 nm. In this way, the total glutathione content in the sample can be determined.

Materials Supplied and Storage Conditions

Kit components	Size		Storage conditions
	48 T	96 T	
Extraction Buffer	50 mL	100 mL	4℃
Assay Buffer	10 mL	20 mL	4℃
GR	6 μL	12 μL	4℃, protected from light
GR Cofactor	1	2	-20℃, protected from light
Chromogen	4 mL	8 mL	4℃, protected from light
Standard	1 (10 mg)	1 (10 mg)	4℃, protected from light

Materials Required but Not Supplied

- Microplate reader or visible spectrophotometer capable of measuring absorbance at 412 nm
- Incubator
- 96-well plate or microglass cuvette, precision pipettes, disposable pipette tips
- Deionized water
- Homogenizer (for tissue samples)

Reagent Preparation

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

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

Diluted GR solution: Before use, add 0.12 mL deionized water into 6 μL GR and equilibrate to room temperature, protected from light. Store at 4℃, protected from light.

Diluted GR Cofactor solution: Add 1.25 mL deionized water into each GR Cofactor and protect from the light. Equilibrate to room temperature before use. Store at -20℃, protected from light.

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

Standard preparation:

Diluted Extraction Buffer: Add 1800 μL deionized water into 200 μL Extraction Buffer.

1 mg/mL GSH Standard: Weigh 1 mg GSH Standard powder, and add 1 mL deionized water.

4 μg/mL GSH Standard: Add 996 μL Diluted Extraction Buffer into 4 μL 1 mg/mL GSH standard.

Dilute the standard furtherly using 4 μg/mL GSH Standard refer to the table below:

Num.	Volume of 4 μg/mL Standard（μL）	Diluted Extraction Buffer（μL）	Concentration (μg/mL)
Std.1	100	0	4
Std.2	50	50	2
Std.3	25	75	1
Std.4	12.5	87.5	0.5
Std.5	5	95	0.2
Std.6	2.5	97.5	0.1
Std.7	1	99	0.04
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 one month.

1. Animal Tissue samples: Weigh 0.1 g tissues, add 1 mL Extraction Buffer and homogenize on ice. Centrifuge at 8,000 g for 10 min at 4℃. Use supernatant for assay, and place it on ice to be tested.

2. Plant Tissue samples: Weigh 0.1 g tissue, add 1 mL Extraction Buffer and mash. 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.

3. Cell or Bacteria: Collect 1×10^6 cells or bacteria into the centrifuge tube, wash cells or bacteria with cold PBS, discard the supernatant after centrifugation; add 1 mL Extraction Buffer to ultrasonically disrupt the cells or bacteria 5 min on ice (power 20% or 200 W, ultrasonic 3 s, interval 7 s, repeat 30 times). Centrifuge at 8,000 g for 10 minutes at 4℃. Use supernatant for assay, and

place it on ice to be tested.

4. Serum: Tested directly.

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. Sample measurement.

Reagent	Blank Well (μL)	Standard Well (μL)	Test Well (μL)
Deionized Water	0	0	18
Diluted Extraction Buffer	20	0	0
Std.	0	20	0
Sample	0	0	2
Assay Buffer	140	140	140
Diluted GR Solution	2	2	2
Diluted GR Cofactor Solution	20	20	20
Chromogen	20	20	20

3. Read the values at 412 nm. A_1 for the first time, and then A_2 for the second time after incubation in darkness for 10 min at 37℃. Finally, calculate $\Delta A = A_2 - A_1$.

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 of standard curve

With the concentration of the standard as the y-axis and the $\Delta\Delta A_{Standard}$ as the x-axis, draw the standard curve.

2. Calculate the T-GSH content

Substitute the $\Delta\Delta A_{Test}$ into the equation to obtain the y value (μg/mL), T-GSH content (μg/mL)**=y×n**

Note: n (the dilution factor) =10. If the $\Delta\Delta A_{Test}$ values of sample are higher than the $\Delta\Delta A_{Standard}$ value of the 200 μg/mL, dilute sample with deionized water and repeat this assay. Multiply the results with the final dilution multiple.

Typical Data

Typical standard curve:

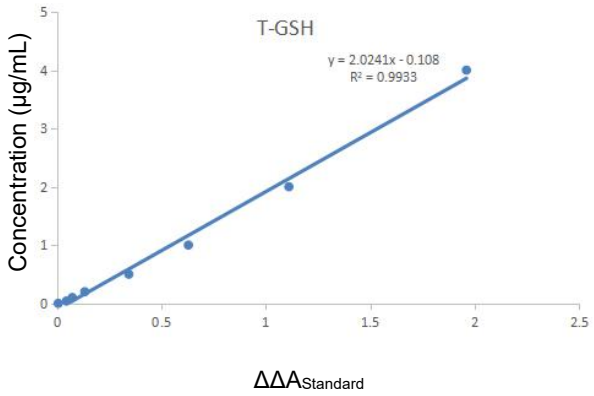

Figure1. Standard curve of T-GSH in 96-well plate assay-data provided for demonstration purposes only. A new standard curve must be generated for each assay.

Recommended Products

Catalog No.	Product Name
KTB1600	CheKine™ Micro Reduced Glutathione (GSH) Assay Kit
KTB1610	CheKine™ Micro Glutathione Oxidized (GSSG) Assay Kit
KTB1620	CheKine™ Micro Glutathione Reductases (GR) Assay Kit
KTB1630	CheKine™ Micro Glutathione S-Transferase (GST) Assay Kit
KTB1640	CheKine™ Micro Glutathione Peroxidase (GSH-Px) Assay Kit
KTB1650	CheKine™ Micro Thioredoxin Reductase (TrxR) Assay Kit
KTB1660	CheKine™ Micro Thioredoxin Peroxidase (TPX) Assay Kit

Disclaimer

The reagent is only used in the field of scientific research, not suitable for clinical diagnosis or other purposes.