CheKine™ Micro Reduced Glutathione (GSH) Assay Kit

Cat #: KTB1600 Size: 48 T/96 T

	Micro Reduced Glutathione (GSH) Assay Kit		
REF	**Cat #:** KTB1600	LOT	**Lot #:** Refer to product label
	Detection range: 2-200 μg/mL		**Sensitivity:** 2 μg/mL
	Applicable samples: Serum, Plasma, Animal/Plant Tissues, Blood Cells, Cells, Bacteria		
	Storage: Stored at 4℃ for 12 months, protected from light		

Assay Principle

Glutathione is a tripeptide of glycine, glutamic acid and cysteine. In the red blood cells, 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 Reduced Glutathione (GSH) Assay Kit provides a simple method for detecting the content of GSH in a variety of biological samples, such as Serum, Plasma, Animal and Plant Tissues, Red Blood cells, Cells, Bacteria. DTNB reacts with reduced glutathione to form a yellow product. The optical density measured at 412 nm, can directly reflect glutathione concentration in the sample.

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℃
Chromogen	4 mL	8 mL	4℃, protected from light
Standard	1	1	4℃, protected from light

Materials Required but Not Supplied

- Microplate reader or visible spectrophotometer capable of measuring absorbance at OD412 nm
- Incubator

- 96-well plate or microglass cuvette, precision pipettes, disposable pipette tips
- Refrigerated centrifuge
- 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℃

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

Standard preparation:

Diluted Extraction Buffer: Extraction Buffer was diluted 10 times with deionized water, in a clean plastic tube by diluting 200 µL Extraction Buffer into 1,800 µL deionized water.

1 mg/mL GSH Standard: Prepare 1 mg/mL GSH Standard by dissolve 1 mg Standard with 1 mL deionized water.

200 µg/mL GSH Standard: Prepare 200 µg/mL of GSH Standard by diluting 200 µL 1 mg/mL GSH Standard into 800 µL Diluted Extraction Buffer. Using 200 µg/mL GSH Standard, prepare standard curve dilution as described in the table in a microplate or microcentrifuge tubes:

Num.	200 µg/mL Standard (µL)	Diluted Extraction Buffer (µL)	Concentration (µg/mL)
Std.1	100	0	200
Std.2	50	50	100
Std.3	25	75	50
Std.4	12.5	87.5	25
Std.5	5	95	10
Std.6	2.5	97.5	5
Std.7	1	99	2
Blank	0	100	0

Notes: Always prepare fresh Standards per use; Diluted Std. 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 Tissues: Weigh 0.1 g tissue, add 1 mL Extraction Buffer and homogenize on ice. Centrifuge at 8,000 g for 10 min at 4°C. Use supernatant for assay, and place it on ice to be tested.

2. Plant Tissues: 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°C. Use supernatant for assay, and place it on ice to be tested.

3. Plasma or Serum: 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.

4. Blood cells: 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 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.

5. Cells or Bacteria: Collect 2×10^6 cells/bacteria for each assay. Wash cells/bacteria with cold PBS twice (resuspend cells/bacteria

with PBS, centrifuge at 600 g for 10 min at 4℃). Resuspend in triple volume of cells/bacteria pellet Extraction Buffer, repeated freeze-thaw cycles 2-3 times (can be frozen in liquid nitrogen, dissolved in 37°C water bath). 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. Add the following reagents to the 96-well plate or microglass cuvette:

Reagent	Blank Well (μL)	Standard Well (μL)	Test Well (μL)
Sample	0	0	20
Deionized Water	20	0	0
Std.	0	20	0
Assay Buffer	140	140	140
Chromogen	40	40	40

3. Mix well, Incubate for 2 min at room temperature, protected from light, record the absorbance value at 412 nm as A_{Blank}, $A_{Standard}$, and A_{Test}.

Note: In order to guarantee the accuracy of experimental results, need to do a pre-experiment with 2-3 samples. If the A value of Samples are higher than the A value of the 200 μg/mL standard, dilute sample with deionized water and repeat this assay. Multiply the results with the dilution factor: n.

Data Analysis

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

1. Drawing the standard curve:

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

2. The GSH concentration of sample is calculated as:

Substitute the ΔA_{Test} into the equation to obtain the y value (μg/mL), The GSH concentration(μg/mL)**=y×n**.

Note: n is dilution factor.

Typical Data

Typical standard curve

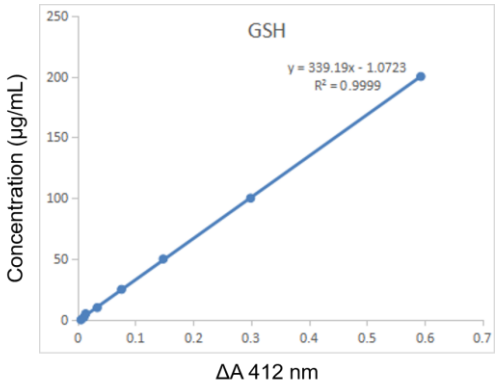

Figure 1. Standard curve of GSH in 96-well plate assay-data provided for demonstration purposes only. A new standard curve must be generated for each assay.

Recommended Products

Catalog No.	Product Name
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

Disclaimer

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