CheKine™ Glutathione Oxidized (GSSG) Colorimetric Assay Kit

Cat #: KTB1610 Size: 96 T

	Glutathione Oxidized (GSSG) Colorimetric 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 comes in two forms: reduced (GSH) and oxidized (GSSG). Glutathione oxidized is the oxidized form of Glutathione, also known as Dithioglutathione, which is formed by the oxidation of two reduced glutathione molecules. GSSG is reduced to GSH by Glutathione Reductase. Reduced glutathione exists in a large number of organisms and plays a major role. Measurement of GSH and GSSG content and GSH/GSSG ratio can reflect cells redox state, and it is also one main indicator of glutathione redox cycle. CheKine™ Glutathione Oxidized (GSSG) Colorimetric Assay Kit provides a simple method for detecting the content of GSSH in a variety of biological samples such as Serum, Plasma, Animal and Plant Tissues, Red Blood cells, Cells, Bacteria. Reduced Glutathione can react with DTNB and generate 2-nitro-5-mercaptobenzoic acid, which has the maximum light absorption at 412 nm wavelength. The original reduced glutathione in the sample is inhibited by 2-vinylpyridine, and then GSSG is reduced to GSH by Glutathione Reductase to determine the content of GSSG.

Materials Supplied and Storage Conditions

Kit components	Size	Storage conditions
	96 T	
Extraction Buffer	100 mL	4℃
Inhibitor	120 μL	-20℃, protected from light
Assay Buffer	20 mL	4℃
GR	12 μL	4℃, protected from light
GR Cofactor	2	-20℃, protected from light
Chromogen	2	4℃, protected from light
Standard	1	4℃, protected from light

Materials Required but Not Supplied

- Microplate Reader capable of measuring absorbance at OD412 nm
- Incubator
- 96-well Plate with clear flat bottom, precision Pipettes, disposable Pipette Tips
- Refrigerated Centrifuge
- Deionized Water
- Dounce homogenizer(for Tissue Samples)

Reagent Preparation

Extracting solution: 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.

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

Diluted GR solution: Add 6 µL GR into 0.12 mL Deionized Water before use. Store at 4℃, protected from light.

Diluted GR Cofactor solution: Add 1.25 mL of Deionized Water into each GR Cofactor before use. Store at -20℃, protected from light.

Diluted Chromogen solution: Add 1.25 mL of Deionized Water into each Chromogen before use. Store at 4℃, 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.

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

20 µM GSSG Standard: Prepare 20 µM of GSSG Standard by diluting 1 µL 20 mM GSSG Standard into 999 µL Diluted Extraction Buffer. Using 20 µM GSSG Standard, prepare standard curve dilution as described in the table in a Microplate or Microcentrifuge tubes:

	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
Blank	0	100	0

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

Sample Preparation

1. Animal Tissues: Weigh 0.1 g Tissues, 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 Tissues, 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. 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℃ 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: The sample can be kept at -80℃ for 10 days. If not do experiments immediately.

Assay Procedure

1. Preheat the Microplate Reader for more than 30 min, and adjust the wavelength to 412 nm.

2. Add the following reagents to the 96-well plate:

Regent	Blank Well (μL)	Standard Well (μL)	Test Well (μL)
Sample	0	0	2
Deionized Water	20	0	18
Different concentrations Standard	0	20	0
Inhibitor	1	1	1
Mix Well, incubate for 30 minutes at 37℃			
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 minutes 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: In order to guarantee the accuracy of experimental results, need to do a pre-experiment with 2-3 samples.

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.

2. The GSSG concentration of Sample is calculated as:

Substitute the $\Delta\Delta A_{Test}$ into the equation to obtain the y value (μM), The GSSG concentration(μM)=y×n.

Note: n is the dilution factor of sample, 10. If the $\Delta\Delta A_{Test}$ value of Samples are higher than the $\Delta\Delta A_{Standard}$ value of the 200 μM Standard, dilute sample with Deionized Water and repeat this assay.

Typical data

Typical standard curve

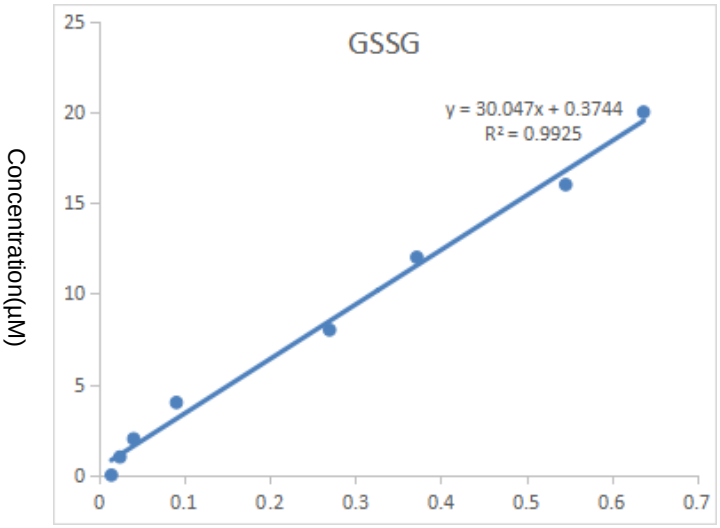

Figure1. Standard Curve of GSSG in 96-well plate assay. Data provided for demonstration purposes only. A new standard Curve must be generated for each assay.

Recommended Products

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

Disclaimer

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