Toal Glutathione (T-GSH) Colorimetric Assay Kit

Cat #: orb1173217 (manual)

Product Features

Product name: Toal Glutathione (T-GSH) Colorimetric Assay Kit

Catalog number: orb1173217

Detection range: 0.04-4 ug/mL
Sensitivity: 0.04 ug/mL

Sample Types: 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 maintaining 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. Toal Glutathione (T-GSH) Colorimetric 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.

Kit Components

Kit components	Size		Storage conditions
	48 T	96 T	
Extraction Buffer	70 mL	70 mL×2	4℃
Assay Buffer	10 mL	20 mL	4℃
GR	7 µL	14 µL	4℃, protected from light
GR Cofactor	Powder×1 vial	Powder×2 vials	-20℃, protected from light
Chromogen	4 mL	8 mL	4℃, protected from light
Standard	Powder×1 vial (10 mg)	Powder×1 vial (10 mg)	4℃, protected from light

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

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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°C.

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

Diluted GR solution: Before use, according to the number of samples, the ratio of adding 1 µL GR to 20 µL deionized water is freshly prepared, mix well.

Diluted GR Cofactor solution: Add 1.5 mL deionized water into each GR Cofactor and protect from the light. Equilibrate to room temperature before use. Store at -20°C for 1 month, protected from light.

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

Standard preparation:

Diluted Extraction Buffer: Add 1800 µL deionized water into 200 µL Extraction Buffer, Diluted Extraction Buffer is used to dilute standard samples.

1 mg/mL GSH Standard: Weigh 1 mg GSH Standard powder and add 1 mL deionized water. Store and aliquot at -20°C for 1 month, protected from light.

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°C 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°C. 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°C. 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°C. Use supernatant for assay and place it on ice to be tested.

4. Serum: Tested directly.

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 # orb1147876, 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 96-well plate or microglass cuvette, follow the table below to set the test:

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. A1 for the first time, and then A2 for the second time after incubation in darkness for 10 min at 37°C. Finally, calculate ΔA =A2-A1.

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Note: To guarantee the accuracy of experimental results, need to do a pre-experiment with 2-3 samples. If the ΔA_{Test} is less than 0.01, the sample size can be appropriately increased. If the $\Delta\Delta A_{Test}$ values of sample are higher than the $\Delta\Delta A_{Standard}$ value of the 4 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, $\Delta\Delta A_{Standard}=\Delta A_{Standard}-\Delta A_{Blank}$, $\Delta\Delta A_{Test}=\Delta A_{Test}-\Delta A_{Blank}$.

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.

Typical Data

Typical standard curve:

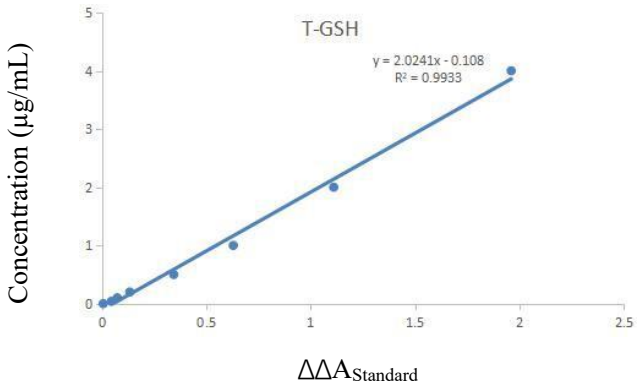

Figure1. Standard curve of T-GSH in 96-well plate assay-data provided for demonstration purposes only. A new standard curve must be generated for each assay.

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.

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