Reduced Glutathione (GSH) Content Assay Kit - Microplate Method

Product code: 112876

Product Introduction

GSH is the major intracellular antioxidant thiol and plays an important role in antioxidation, protein thiol protection, amino acid transmembrane transport, and other processes. The reduced/oxidized ratio (GSH/GSSG) is a main dynamic indicator of the cellular redox state. Measuring intracellular GSH and GSSG levels and the GSH/GSSG ratio can effectively reflect the redox state of cells.

DTNB reacts with GSH to form a complex with a characteristic absorption peak at 412 nm. Its absorbance is proportional to the GSH content.

Example reference result: sample tested was pig liver. OD412 nm: blank 0.117; measurement 0.324/0.319/0.323. Actual readings may vary depending on the instrument and test conditions.

Product Packing List

Item	Description	Volume	Storage
BR5000373.1	Reagent I	100 mL	2-8 °C
BR5000373.2	Reagent II	14 mL	2-8 °C
BR5000373.3	Reagent III	5 mL	Protect from light, 2-8 °C
BR5000373.4	Standard	3.1 mg	2-8 °C
BR5000373.m	Instruction Manual	1 copy	/

Package size: 100T

Quality Standards and Safety Instructions

Raw Material and Packaging Name	Quality Standard	Main Toxicity
Reagent I	—	—
Reagent II	—	—
Reagent III	—	—
Standard	—	—

Transport and Storage Conditions

Transport this product with ice packs.

Store according to the instructions above. Shelf life: 180 days.

Product Instructions

1. Crude Enzyme Extract Preparation

1.1 Tissue: Use a tissue mass (g) to Reagent I volume (mL) ratio of 1:5-10. It is recommended to weigh about 0.1 g tissue and add 1 mL Reagent I, then homogenize in an ice bath. Centrifuge at 12000g, 4 °C for 10 min. Collect the supernatant and keep it on ice for testing.

1.2 Bacteria and fungi: Use a ratio based on cell number (10^4cells): Reagent I: anhydrous ethanol volume (mL) = 1000-2000:1:1. Recommended: for 500 x 10^4cells, add 0.5 mL Reagent I and 0.5 mL anhydrous ethanol. Disrupt cells ultrasonically in an ice bath at 300 W, sonicate for 3 s with 7 s intervals, total time 3 min. Then incubate at 40 °C for 40 min, centrifuge at 12000g for 10 min, collect the supernatant, and place it on ice for testing.

1.3 Serum (plasma), culture medium, and other liquid samples: Pipette 100 µL of liquid sample, add 100 µL Reagent I, and mix well. Centrifuge at 4 °C and 12000g for 10 min. Collect the supernatant and place it on ice for testing.

2. Preparation of Solutions

Standard: Add 1 mL distilled water to dissolve the standard and prepare a 10 mmol/L standard solution. Store at 2-8 °C for up to 6 weeks.

3. Assay Procedure

1. Preheat the microplate reader for 30 min, set the wavelength to 412 nm, and zero with distilled water.
2. Preheat Reagent II at 37 °C for 10 min.
3. Dilute the 10 mmol/L standard solution with distilled water to prepare 2500, 1250, 625, 156.25, 39.06, and 19.53 µmol/L standard solutions for assay.

4. Standard Solution Dilution Table

No.	Concentration Before Dilution (µmol/L)	Standard Solution Volume (µL)	Distilled Water Volume (µL)	Concentration After Dilution (µmol/L)
1	10000	15	45	2500
2	2500	25	25	1250
3	1250	20	20	625
4	625	15	45	156.25
5	156.25	15	45	39.06
6	39.06	20	20	19.53

Each standard tube in the following experiment requires 20 µL standard solution. Do not directly measure absorbance in this step.

5. Procedure Table

Component	Blank Tube	Test Tube	Standard Tube
Distilled water (µL)	20	-	-
Sample (µL)	-	20	-
Standard solution (µL)	-	-	20
Reagent II (µL)	140	140	140
Reagent III (µL)	40	40	40

Mix well and let stand at room temperature for 5 min. Measure the absorbance of the blank tube, test tube, and standard tube at 412 nm. Record them as A_{blank}, $A_{measurement}$, and $A_{standard}$. Calculate:

$\Delta A = A_{measurement} - A_{blank}$

$\Delta A_{standard} = A_{standard} - A_{blank}$

The blank tube and the standard curve only need to be measured once or twice.

6. GSH Content/Activity Calculation

6.1 Construction of the standard curve: Use the concentration of the standard tube (X, µmol/L) and the absorbance $\Delta A_{standard}$(Y, $\Delta A_{standard}$) to establish the standard curve. Substitute the sample ΔA (Y, ΔA) into the standard curve formula to calculate the sample concentration (X, µmol/L).

6.2 GSH content calculation:

Calculated by protein concentration:

GSH (μmol/mg prot) = X x V_{sample}÷ (V_{sample}x Cpr) x 10^{-3}= 10^{-3}x X ÷ Cpr

Calculated by sample fresh weight:

GSH (μmol/g fresh weight) = X x V_{sample}÷ (V_{sample}÷ $V_{total\ sample}$x W) x 10^{-3}= 10^{-3}x X ÷ W

By cell/bacterial count calculation:

GSH (μmol/10^4cell) = X x V_{sample}÷ (V_{sample}÷ $V_{total\ sample}$x cells/bacterial count) x 10^{-3}= 10^{-3}x X ÷ cell count

By liquid volume:

GSH (μmol/mL) = X x (V_{liquid}+ $V_{Reagent\ I}$) ÷ V_{liquid}x 10^{-3}= 10^{-3}x 2 x X

Definitions:

- $V_{total\ sample}$: total volume of supernatant, 1 mL
- V_{sample}: volume of supernatant added to the reaction system, 20 μL = 0.02 mL
- Cpr: protein concentration of the supernatant, mg/mL
- W: sample mass, g
- 10^{-3}: 1 mL = 10^{-3}L
- V_{liquid}: volume of liquid sample added during extraction, 0.1 mL
- $V_{Reagent\ I}$: volume of Reagent I added during extraction, 0.1 mL
- Cells/Bacterial count: in units of 10,000

Precautions

1. Before formal determination, select 2-3 samples with large expected differences for pretesting. This 100T kit can test 96 samples.
2. Required instruments and supplies: anhydrous ethanol, microplate reader, analytical balance, homogenizer/mortar/cell ultrasonic disruptor, refrigerated centrifuge, water bath, adjustable pipette, 96-well plate, ice, and distilled water.
3. Reagent I contains a protein precipitant, so the supernatant cannot be used for protein concentration measurement. If protein content needs to be determined, use separate tissue.
4. Linear range of this kit: 0-2500 μmol/L.
5. This product is for scientific research use by professionals only. It must not be used for clinical diagnosis or treatment, must not be used for food or drugs, and must not be stored in ordinary residences.
6. Wear a lab coat and disposable gloves during operation.

Appendix

> For greater accuracy, the standard curve should be prepared by the user. Refer to the procedure table above. The user may use the standard curve formula or the absorbance values of each standard well obtained according to the procedure table to plot a standard curve (R^2≥ 0.99) and obtain the calculation formula for sample calculation.

Visual Reference