Reduced Glutathione (GSH) Content Assay Kit - Spectrophotometric Method

Product Code: 112870

Product Introduction

GSH is the major intracellular antioxidant sulfhydryl substance and plays important roles in antioxidation, protection of protein sulfhydryl groups, and transmembrane transport of amino acids. The ratio of reduced to oxidized glutathione (GSH/GSSG) is an important dynamic indicator of the cellular redox state. Measuring intracellular GSH and GSSG contents, as well as 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. The absorbance is proportional to the GSH content.

Example result: Sample tested: porcine liver. OD412 nm: Blank 0.115; Test 0.350 / 0.353 / 0.379. Actual readings may vary depending on instrument and test conditions. These data are for reference only.

Package Contents

Item	Description	Volume / Amount	Storage
BR5000289.1	Reagent I	50 mL	2-8 °C
BR5000289.2	Reagent II	36 mL	2-8 °C
BR5000289.3	Reagent III	10 mL	Protect from light, 2-8 °C
BR5000289.4	Standard	3.1 mg	2-8 °C
BR5000289.m	Instructions	1 copy	-

Pack size: 50T

Quality Standards and Safety

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

Transportation and Storage

Transportation: Transport with ice packs.

Storage: Store as instructed for each component.

Shelf life: 180 days.

Instructions for Use

1. Preparation of Crude Enzyme Extract

1.1 Tissue: Homogenize tissue in an ice bath at 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. Centrifuge at 8000 g, 4 °C for 10 min. Collect the supernatant and keep it on ice for testing.

1.2 Bacteria and fungi: Use a ratio of cell number (10^4cells) : Reagent I : absolute ethanol (mL) = 1000-2000:1:1. It is recommended to mix 5 million cells with 0.5 mL Reagent I and 0.5 mL absolute ethanol. Disrupt cells by ultrasonication in an ice bath (power 300 W, sonicate 3 s, interval 7 s, total time 3 min). Then incubate at 40 °C for 40 min, centrifuge at 8000 g for 10 min, collect the supernatant, and keep it on ice for testing.

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

2. Preparation of Solutions

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

3. Determination Procedure

1. Preheat the spectrophotometer for 30 min, set the wavelength to 412 nm, and zero the instrument 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 standards.

4. Standard Dilution Table

No.	Concentration Before Dilution (μmol/L)	Standard Solution Volume (μL)	Distilled Water Volume (μL)	Concentration After Dilution (μmol/L)
1	10000	75	225	2500
2	2500	125	125	1250
3	1250	100	100	625
4	625	75	225	156.25
5	156.25	75	225	39.06
6	39.06	100	100	19.53

Each standard tube in the following experiment requires 100 μL of standard solution. Do not measure absorbance directly at this step.

5. Reaction Setup

Component	Blank Tube	Assay Tube	Standard Tube
Distilled water (μL)	100	-	-
Sample (μL)	-	100	-
Standard solution (μL)	-	-	100
Reagent II (μL)	700	700	700
Reagent III (μL)	200	200	200

Mix well and let stand at room temperature for 5 min. Measure the absorbance of the blank tube, assay tube, and standard tube at 412 nm, recorded as A_{blank}, A_{assay}, and $A_{standard}$.

Calculate:

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

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

The blank tube and standard curve only need to be measured one to two times.

Calculation of GSH Content

1. Standard Curve

Establish the standard curve using the standard tube concentration (X, μmol/L) and absorbance $\Delta A_{standard}$ (Y, $\Delta A_{standard}$). Use the standard curve and substitute ΔA (Y, ΔA) into the formula to calculate the sample concentration (X, μmol/L).

2. Calculation Formulas

By protein concentration:
GSH (μmol/mg prot) = $X \times V_{sample} \div (V_{sample} \times Cpr) \times 10^{-3} = 10^{-3} \times X \div Cpr$

By fresh sample weight:
GSH (μmol/g fresh weight) = $X \times V_{sample} \div (V_{sample} \div V_{total\ sample} \times W) \times 10^{-3} = 10^{-3} \times X \div W$

By cell or bacterial count:
GSH (μmol/10^4cell) = $X \times V_{sample} \div (V_{sample} \div V_{total\ sample} \times$ cell/bacterial count$) \times 10^{-3} = 10^{-3} \times X \div$ cell count

By liquid volume:
GSH (μmol/mL) = $X \times (V_{liquid} + V_{reagent}) \div V_{liquid} \times 10^{-3} = 10^{-3} \times 2 \times X$

3. Parameter Definitions

- $V_{total\ sample}$: total supernatant volume, 1 mL
- V_{sample}: volume of supernatant added to the reaction system, 100 μL = 0.1 mL
- W: sample mass, g
- Cpr: supernatant protein concentration, mg/mL
- 10^{-3}: 1 mL = 10^{-3}L
- V_{liquid}: liquid sample volume used during extraction, 0.1 mL
- $V_{reagent}$: volume of Reagent I added during extraction, 0.1 mL
- Cells / number of bacteria: expressed in ten thousands

Precautions

1. Before the formal assay, select 2-3 samples with large expected differences for pre-testing. This 50T kit can test 48 samples.
2. Required instruments and supplies not provided: anhydrous ethanol, visible spectrophotometer, analytical balance, homogenizer, mortar, cell ultrasonic disruptor, refrigerated centrifuge, water bath, adjustable pipette, 1 mL glass cuvette, ice, and distilled water.
3. Reagent I contains a protein precipitant, so the supernatant cannot be used for protein concentration determination. If protein content must be measured, prepare a separate tissue sample.
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, or for food or pharmaceuticals, and must not be stored in an ordinary residence.
6. Wear a lab coat and disposable gloves during operation.

Appendix

For greater accuracy, prepare the standard curve during use. Follow the operation table above. You may use the resulting standard curve formula, or plot a standard curve from the absorbance values of each standard tube obtained according to the procedure table ($R^2 \geq 0.99$) to calculate sample results.

Visual Reference