Non-Protein Sulfhydryl Content Assay Kit

Product Code: 112905

Non-Protein Sulfhydryl Content Assay Kit - Micro Method

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Product Introduction

Thiol groups in organisms mainly include non-protein thiols and protein thiols. Thiol compounds have important detoxification functions in vivo and are physiologically significant for self-regulation.

The thiol group reacts with 5,5'-dithiobis-(2-nitrobenzoic acid) (DTNB) to form a yellow compound with a maximum absorption peak at 412 nm.

Reference result: 2x diluted pig liver sample. OD412 nm: Control 0.064; Sample 0.314/0.323. Actual readings may vary depending on the instrument and test conditions. These data are for reference only.

Package Contents

Item Code	Reagent	Quantity	Storage
112905.1	Reagent I	20 mL	2-8°C
112905.2	Reagent II	0.75 mL	Protect from light, 2-8°C
112905.3	Extraction Solution	110 mL	Protect from light, 2-8°C
112905.4	Standard	10 mg	Protect from light, 2-8°C
112905.m	Manual	1 copy	-

Pack size: 100T

Quality Standards and Safety Instructions

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

Transportation and Storage

Transportation: Transport with ice packs.

Storage: Store according to the instructions above.

Shelf life: 180 days.

Instructions for Use

1. Sample Preparation

1. **Tissue samples:** Extract according to a tissue mass (g) : extraction solution volume (mL) ratio of 1:5-10. It is recommended to weigh about 0.1 g tissue and add 1 mL extraction solution. Homogenize in an ice bath, then centrifuge at 8000g and 4°C for 10 min. Collect the supernatant and keep it on ice for testing.

2. **Serum or culture medium:** Take 0.5 mL sample and add 0.5 mL extraction solution. Mix well, let stand at room temperature for 10 min, then centrifuge at 8000g and 4°C for 10 min. Collect the supernatant and keep it on ice for testing.

2. Reagent Preparation

Standard: Dissolve 10 mg reduced glutathione in 1.3 mL extraction solution to prepare a 25 μmol/mL standard solution. Store at 2-8°C for 4 weeks.

3. Procedure

1. Preheat the spectrophotometer or microplate reader for 30 min. Set the wavelength to 412 nm and zero with distilled water.
2. Dilute the 25 μmol/mL standard solution with extraction solution to prepare standards of 1, 0.5, 0.25, 0.125, 0.0625, 0.03125, 0.015625, and 0.0078125 μmol/mL.

No.	Concentration Before Dilution (μmol/mL)	Standard Solution Volume (μL)	Extraction Solution Volume (μL)	Concentration After Dilution (μmol/mL)
1	25	8	192	1
2	1	100	100	0.5
3	0.5	100	100	0.25
4	0.25	100	100	0.125
5	0.125	100	100	0.0625
6	0.0625	100	100	0.03125
7	0.03125	100	100	0.015625
8	0.015625	100	100	0.0078125

Each standard tube in the following experiment requires 40 μL standard solution. Do not directly measure absorbance at this step.

4. Assay Procedure

Component	Control Tube	Assay Tube	Standard Tube	Blank Tube
Sample (μL)	40	40	-	-
Standard (μL)	-	-	40	-
Distilled Water (μL)	-	-	-	40
Reagent I (μL)	150	150	150	150
Reagent II (μL)	-	10	10	-
Anhydrous Ethanol (μL)	10	-	-	10

Mix well and let stand at 25°C for 10 min. Measure absorbance at 412 nm and record as Acontrol, Aassay, Astandard, and Ablank.

Calculate ΔA = Aassay - Acontrol and ΔAstandard = Astandard - Ablank.

Each assay tube is provided with one control tube. The standard curve and blank tube only need to be measured 1-2 times.

Calculation of Non-Protein Sulfhydryl Content

1. Standard Curve

Use the standard tube concentration (X, μmol/mL) and absorbance (Y, ΔAstandard) to establish a standard curve. Then substitute the sample ΔA (Y, ΔA) into the curve formula to calculate the sample concentration X (μmol/mL).

2. Calculation Formulas

Calculated by sample mass: Non-protein sulfhydryl content (μmol/g mass) = X × Vextract ÷ W × F = X ÷ W × F

Calculated by serum (plasma) or other liquid volume: Non-protein sulfhydryl content (μmol/mL) = X × (Vliquid extract + Vliquid) ÷ Vliquid × F = 2 × X × F

Calculated by protein concentration: Non-protein sulfhydryl content (μmol/mg prot) = X × Vextract ÷ (Cpr × Vextract) × F = X ÷ Cpr × F

- Vextract: total volume of sample extract, 1 mL
- W: sample mass, g
- Cpr: sample protein concentration, mg/mL
- Vliquid extract: total volume of liquid sample extract, 0.5 mL
- Vliquid: volume of serum (plasma) or other liquid, 0.5 mL
- F: sample dilution factor

Precautions

1. Before the formal assay, select 2-3 samples with large expected differences for a pretest. This 100T kit can test 48 samples.
2. Required instruments and supplies: benchtop centrifuge, visible spectrophotometer or microplate reader, constant-temperature water bath, micro glass cuvette or 96-well plate, adjustable pipette, mortar or homogenizer, anhydrous ethanol, ice, and distilled water.
3. The linear detection range of this kit is 0.0078125-1 μmol/mL.
4. If the measured absorbance exceeds the linear range, increase the sample amount or dilute the sample before measurement.
5. The extract contains a protein precipitant, so the supernatant cannot be used for protein concentration determination. If protein content needs to be measured, use separate tissue.

Appendix

> For greater accuracy, the standard curve should be prepared by the customer. Based on the operation table above, use either the standard curve formula or the absorbance values of each standard well to plot a standard curve ($R^2 \geq 0.99$) and obtain the calculation formula for sample analysis.