

112904 Non-Protein Thiol Content Assay Kit**Product Introduction**

Thiols in organisms mainly include non-protein thiols and protein thiols. Thiol compounds have important detoxification functions in vivo and are of great physiological significance for the self-regulation of organisms.

Thiol groups react with 5,5'-dithio-bis-nitrobenzoic acid (DTNB) to produce a yellow compound with a maximum absorption peak at 412 nm.

Reference result: 2-fold diluted pig liver sample. OD412 nm: control 0.097; sample 0.492/0.497. Actual readings may vary under different test conditions and with different instruments. These data are for reference only.

Packing List

Code	Component	Volume	Storage
112904.1	Reagent I	40 mL	2-8°C
112904.2	Reagent II	1.5 mL	Protected from light, 2-8°C
112904.3	Extraction Solution	60 mL	Protected from light, 2-8°C
112904.4	Standard	10 mg	Protected from light, 2-8°C
112904.m	Manual	1 copy	-

Pack size: 50T

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 this product with ice packs.

Storage: Store according to the instructions above.

Shelf life: 180 days.

Instructions for Use**1. Sample Preparation**

1. Tissue sample extraction: The ratio of tissue mass (g) to extraction solution volume (mL) should be 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 and let stand at room temperature for 10 min.

Centrifuge at 8000g and 4°C for 10 min. Collect the supernatant and keep it on ice for testing.

2. Reagent Preparation

Standard: 10 mg reduced glutathione.

Before use, add 1.3 mL extraction solution to dissolve it into a 25 $\mu\text{mol/mL}$ standard solution. Store at 2-8°C for 4 weeks.

3. Procedure

1. Preheat the spectrophotometer for 30 min, set the wavelength to 412 nm, and use distilled water to zero the instrument.
2. Dilute the 25 $\mu\text{mol/mL}$ standard solution with extraction solution to prepare 0.5, 0.25, 0.125, 0.0625, 0.03125, 0.015625, and 0.0078125 $\mu\text{mol/mL}$ standard solutions.

Standard Solution Dilution Table

No.	Concentration Before Dilution ($\mu\text{mol/mL}$)	Standard Solution Volume (μL)	Extraction Solution Volume (μL)	Concentration After Dilution ($\mu\text{mol/mL}$)
1	25	20	980	0.5
2	0.5	500	500	0.25
3	0.25	500	500	0.125
4	0.125	500	500	0.0625
5	0.0625	500	500	0.03125
6	0.03125	500	500	0.015625
7	0.015625	500	500	0.0078125

Each standard tube in the following experiment requires 200 μL standard solution. Do not directly measure absorbance in this step.

4. Assay Procedure

Assay method: spectrophotometric method for non-protein sulfhydryl content.

Component	Control Tube	Test Tube	Standard Tube	Blank Tube
Sample (μL)	200	200	-	-
Standard (μL)	-	-	200	-
Distilled water (μL)	-	-	-	200
Reagent I (μL)	750	750	750	750
Reagent II (μL)	-	50	50	-
Anhydrous ethanol (μL)	50	-	-	50

Mix well, let stand at 25°C for 10 min, then measure absorbance at 412 nm.

Record the absorbance as A_{control} , A_{assay} , A_{standard} , and A_{blank} .

Calculate $\Delta A = A_{\text{assay}} - A_{\text{control}}$ and $\Delta A_{\text{standard}} = A_{\text{standard}} - A_{\text{blank}}$.

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

5. Calculation of Non-Protein Sulfhydryl Content

5.1 Standard Curve

Plot a standard curve using standard concentration (X, $\mu\text{mol/mL}$) and absorbance (Y, $\Delta A_{\text{standard}}$). Use the standard curve to convert the

sample ΔA value to sample concentration X ($\mu\text{mol/mL}$).

5.2 Calculation Formulas

Calculated based on sample mass:

$$\text{Non-protein sulfhydryl content } (\mu\text{mol/g mass}) = X \times V_{\text{extraction}} \div W \times F = X \div W \times F$$

Calculated based on serum (plasma) or other liquid sample volume:

$$\text{Non-protein sulfhydryl content } (\mu\text{mol/mL}) = X \times (V_{\text{liquid extraction}} + V_{\text{liquid}}) \div V_{\text{liquid}} \times F = 2 \times X \times F$$

Calculated based on protein concentration:

$$\text{Non-protein sulfhydryl content } (\mu\text{mol/mg prot}) = X \times V_{\text{extraction}} \div (C_{\text{pr}} \times V_{\text{extraction}}) \times F = X \div C_{\text{pr}} \times F$$

Symbol	Definition	Value / Unit
Vextraction	Total volume of sample extract	1 mL
W	Sample mass	g
Cpr	Sample protein concentration	mg/mL
Vliquid extraction	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 pretesting. This 50T kit can test 24 samples.
2. Required instruments and supplies to be provided by the user: benchtop centrifuge, visible spectrophotometer, constant-temperature water bath, 1 mL glass cuvette, adjustable pipette, mortar/homogenizer, anhydrous ethanol, ice, and distilled water.
3. The linear detection range of this kit is 0.0078125-0.5 $\mu\text{mol/mL}$.
4. If the measured absorbance exceeds the linear range, the sample amount can be increased or the sample can be diluted 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 determined, a separate tissue sample is required.

Appendix

The standard curve is more accurate when 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 prepare a standard curve ($R^2 \geq 0.99$) and obtain the calculation formula for sample calculation.