



Thiol Microplate Assay Kit

User Manual

Catalog # ASK1159

Detection and Quantification of Thiol Content in Urine, Serum, Plasma, Tissue extracts, Cell lysate, Cell culture media, Other biological fluids Samples.

For research use only. Not for diagnostic or therapeutic procedures.

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I. INTRODUCTION

Thiol groups, found as free cysteine, glutathione (GSH), and cysteine residues in proteins, are involved in many biological processes. The disulfide bonds generated when the thiol groups of two cysteine residues are oxidized contribute to the tertiary or quaternary structure of a protein.

Thiol Microplate Assay Kit is a sensitive assay for determining Thiol concentration in various samples. The reaction products can be measured at a colorimetric readout at 412 nm.



II. KIT COMPONENTS

Component	Volume	Storage
96-Well Microplate	1 plate	
Assay Buffer	30 ml x 4	4 °C
Reaction Buffer	10 ml x 1	4 °C
Dye Reagent	Powder x 1	4 °C
Standard	1 ml x 1	4 °C
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Note:

Dye Reagent: add 2 ml Assay Buffer to dissolve before use.

Standard: add 1 ml Assay Buffer to dissolve, then add 0.1 ml into 0.9 ml Assay Buffer, the concentration will be 2 mmol/L.

III. MATERIALS REQUIRED BUT NOT PROVIDED

1. Microplate reader to read absorbance at 412 nm
2. Distilled water
3. Pipettor
4. Pipette tips
5. Mortar
6. Centrifuge
7. Timer



IV. SAMPLE PREPARATION

1. For serum, plasma, cells lysate or other liquid samples

Add 0.1 ml sample into 0.9 ml Assay Buffer, centrifuged at 8000g 4 °C for 10 minutes, take the supernatant into a new centrifuge tube for detection.



V. ASSAY PROCEDURE

Add following reagents into the microcentrifuge tubes:

Reagent	Sample	Standard	Blank
Sample	20 μ l	--	--
Standard	--	20 μ l	--
Distilled water	--	--	20 μ l
Reaction Buffer	100 μ l	100 μ l	100 μ l
Dye Reagent	20 μ l	20 μ l	20 μ l
Mix, record absorbance measured at 412 nm.			



VI. CALCULATION

1. According to the volume of sample

$$\begin{aligned} \text{Thiol (mmol/L)} &= C_{\text{Standard}} \times V_{\text{Standard}} \times (\text{OD}_{\text{Sample}} - \text{OD}_{\text{Blank}}) / (\text{OD}_{\text{Standard}} - \text{OD}_{\text{Blank}}) / V_{\text{Sample}} \\ &\quad \times 10 \\ &= 20 \times (\text{OD}_{\text{Sample}} - \text{OD}_{\text{Blank}}) / (\text{OD}_{\text{Standard}} - \text{OD}_{\text{Blank}}) \end{aligned}$$

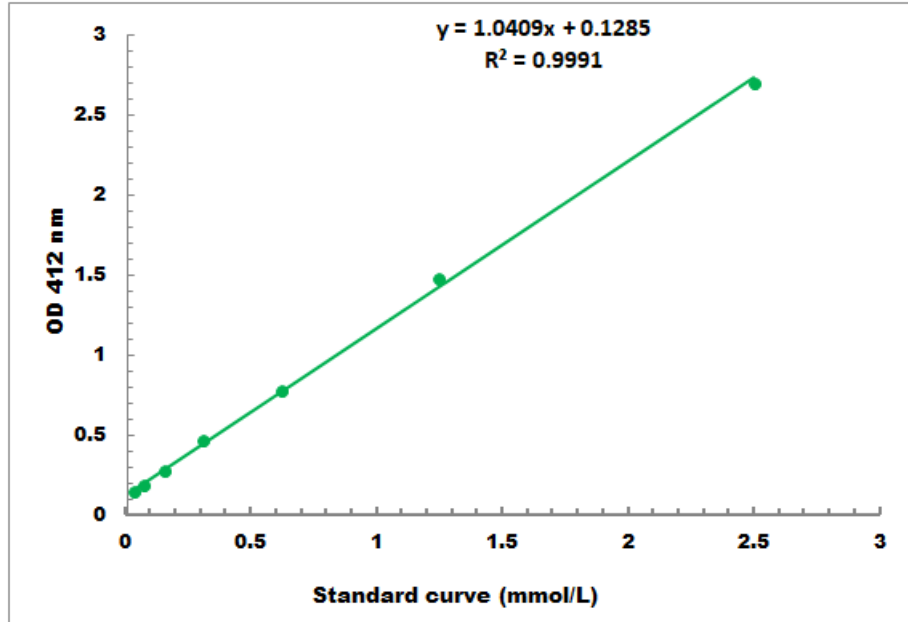
C_{Standard} : the standard concentration, 2 mmol/L;

V_{Standard} : the volume of standard, 0.02 ml;

V_{Sample} : the volume of sample, 0.02 ml.

VII. TYPICAL DATA

The standard curve is for demonstration only. A standard curve must be run with each assay.



Detection Range: 0.02 mmol/L - 2 mmol/L