Thiol Microplate Assay Kit

User Manual

Catalog # FTA0143

(Version 1.2A)

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.

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 quatenary 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, multi-channel 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 µl	--	--
Standard	--	20 µl	--
Distilled water	--	--	20 µl
Reaction Buffer	100 µl	100 µl	100 µl
Dye Reagent	20 µl	20 µl	20 µl
Mix, record absorbance measured at 412 nm.			

Note:

1) Perform 2-fold serial dilutions of the top standards to make the standard curve.

2) The concentrations can vary over a wide range depending on the different samples. For unknown samples, we recommend doing a pilot experiment & testing several doses to ensure the readings are within the standard curve range.

VI. CALCULATION

1. According to the volume of sample

$$\text{Thiol (mmol/L)} = C_{Standard} \times V_{Standard} \times (OD_{Sample} - OD_{Blank}) / (OD_{Standard} - OD_{Blank}) / V_{Sample} \times 10$$

$$= 20 \times (OD_{Sample} - OD_{Blank}) / (OD_{Standard} - OD_{Blank})$$

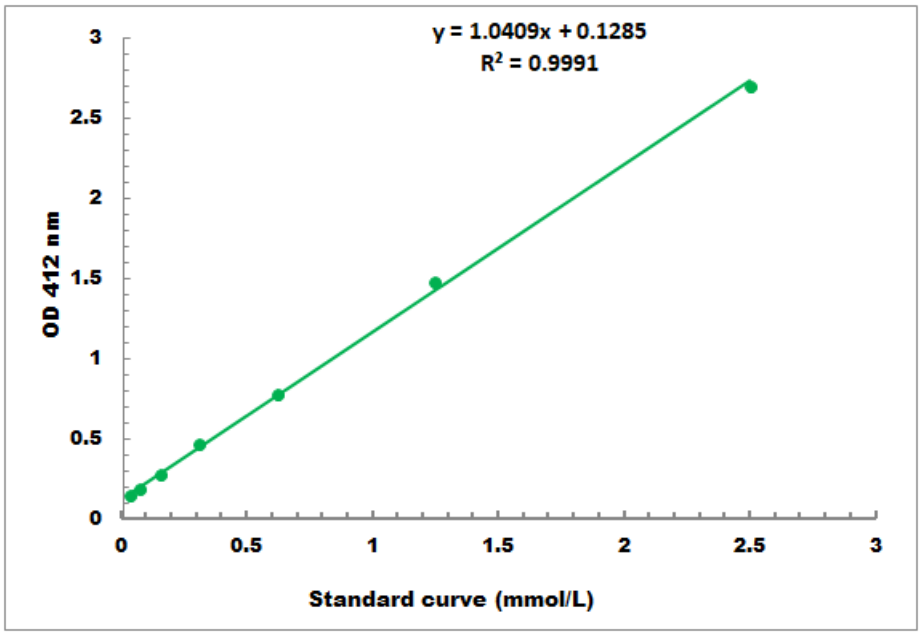
$C_{Standard}$: the standard concentration, 2 mmol/L;

$V_{Standard}$: 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

VIII. TECHNICAL SUPPORT

For troubleshooting, information or assistance, please go online to www.cohesionbio.com or contact us at techsupport@cohesionbio.com

IX. NOTES