Thiol

Colorimetric Microplate Assay Kit

User Manual

Catalog # CAK1159

(Version 1.4A)

Detection and Quantification of ThiolContentin Urine, Serum, Plasma, Tissue extracts, Cell lysate, Cell culture media, Other biological fluidsSamples.

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 quaternary structure of a protein.

ThiolColorimetric Microplate Assay Kit is a sensitive assay for determining Thiolconcentration 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	30mlx 4	4 °C
Reaction Buffer	10 ml x 1	4 °C
Dye Reagent	Powder x 1	4 °C, keep in dark
Standard	1 ml x 1	4 °C
Technical Manual	1 Manual	

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. REAGENT PREPARATION

Dye Reagent: Briefly centrifuge prior to opening.Add 2 ml Assay Bufferto dissolve before use.Keep in dark and store at 4°C for 2weeks or -20 °C for 3 months.

Standard: Briefly centrifuge prior to opening. Dissolvein1 ml Assay Bufferto generate 20 mmol/L of standardstock solution, store at -20°C for 1-2weeksafter reconstitution.Then dilute to2 mmol/Lstandard top solutionby adding0.1 ml stock solution into 0.9 ml Assay Buffer.Perform 2-fold serial dilutions of the top standardsolution using Assay Bufferto make the standard curve.The concentration of standard curve could be2/1/0.5/0.25/0.125/0.0625/0.0312mmol/L.

V. SAMPLE PREPARATION

1.For serum,plasma, cells lysateor other liquidsamples

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.

VI. ASSAY PROCEDURE

Add following reagents intothe microplate:

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

Note:

*Reagents must be added sequentially and should not be premixed prior to addition.

**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.

**For colored samples, we recommend setting a parallel sample background control well with same volume of sample only. Other reagents were replaced by distilled water to the same total volume. Subtract the OD value of the sample background control from the OD value of the sample to correct for interference from the sample's own color.

VII. CALCULATION

1. Calculatethe sample concentration in ASSAY PROCEDURE according to the slope of the standard curve

$$C=\frac{(OD_{Sample}-OD_{Blank})-\text{Intercept}}{\text{Slope}}\times n(mmol/L)$$

Calculate the initial concentration according to sample preparation procedure.

2. According to one point of the standard OD and concentration

2.1According to the volume of sample

$$C=\frac{(C_{Standard}\times V_{Standard})\times(OD_{Sample}-OD_{Blank})}{(OD_{Standard}-OD_{Blank})\times V_{Sample}}\times\frac{V_{Assay}+V}{V}(\mu mol/ml)$$

Slope: the absorbance slope of standard curve

n: the dilution factor

$C_{Standard}$: the standard concentration, mmol/L = μmol/ml

$V_{Standard}$: the volume of standard in assay procedure, μl

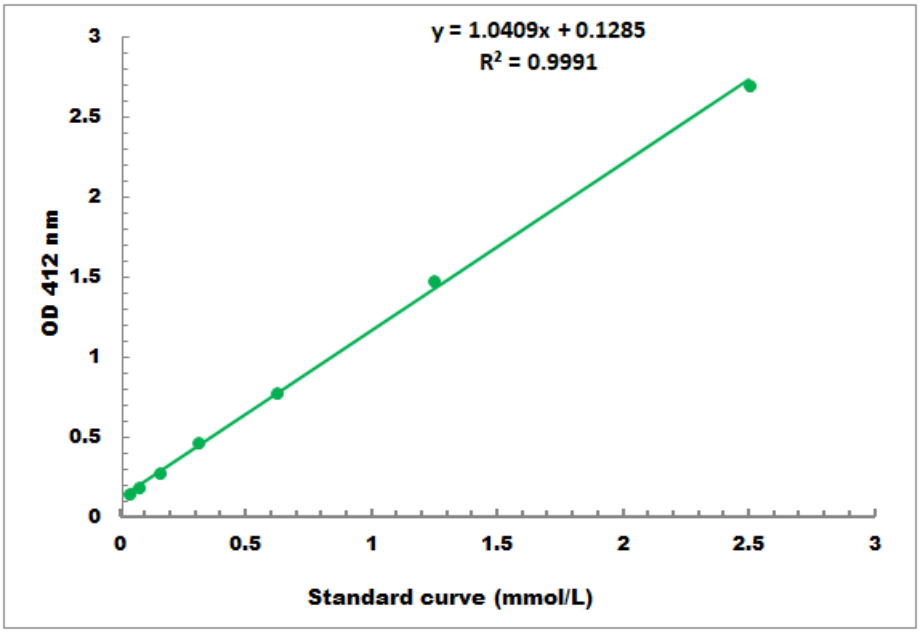
V_{Sample}: the volume of sample in assay procedure, μl

V_{Assay}: the volume of Assay Buffer, μl

V: the volume of sample in sample preparation, μl

VIII. 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