Thiol Microplate Assay Kit

Instructions for Use | Cat. No.: ARS6754

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 .

1. 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 Colorimetric 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.

2. 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, keep in dark
Standard	1 mL x 1	4 °C
Technical Manual	1 Manual	-

3. 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

4. Reagent Preparation

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

Standard: Briefly centrifuge prior to opening. Dissolve in 1 mL Assay Buffer to generate 20 mmol/ L of standard stock solution , store at -20°C for 1-2 weeks after reconstitution. Then dilute to 2 mmol/ L standard top solution by adding 0.1 mL stock solution into 0.9 mL Assay Buffer. Perform 2 -fold serial dilutions of the top standard solution using Assay Buffer to make the standard curve. The concentration of standard curve could be 2/1/0.5/0.25/0.125/0.0625/0.0312 mmol/ L.

5. 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.

6. Assay Procedure

Add following reagents in to the 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	20 µl	20 µl	20 µl
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.

7. Calculation

1. Calculate the sample concentration in ASSAY PROCEDURE according to the slope of

the standard curve

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

Calculate the initial concentration according to sample preparation procedure .

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

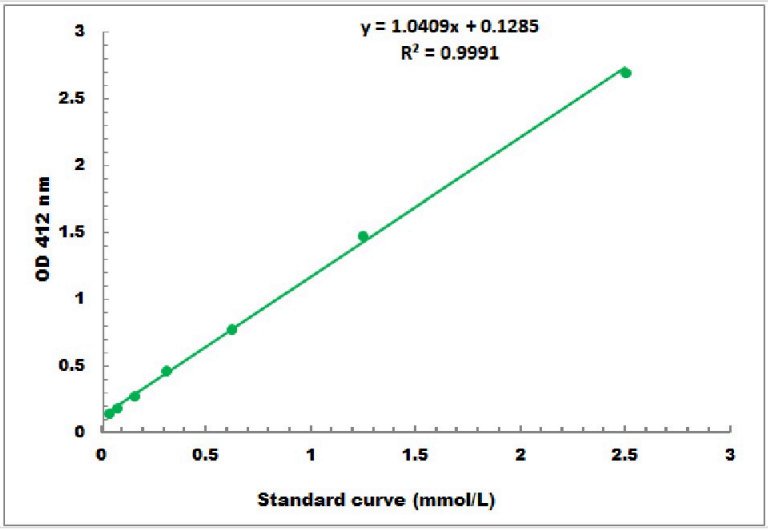
2.1 According to the volume of sample

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

Item	Definition
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

8. 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