



Cysteine

Colorimetric Microplate Assay Kit

User Manual

Catalog # CAK1133

(Version 1.3A)

Detection and Quantification of Cysteine Content in Tissue extracts,
Cell lysate, Cell culture media and Other biological fluids Samples.

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

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I. INTRODUCTION

Cysteine (CYS) is a sulfhydryl-containing amino acid and an important structural and functional part of proteins. In animals, cysteine is synthesized from transsulfuration of homocysteine, which is itself derived from metabolism of the amino acid methionine. Cystathionine β -synthase catalyzes condensation of homocysteine with serine to form cystathionine, which is deaminated and hydrolyzed by Cystathionine β -lyase to form cysteine and α -ketobutyrate. Because of its nucleophilic nature, the thiol group of cysteine has numerous biological functions. The formation of disulfide linkages between the thiol groups of cysteine residues helps to stabilize the tertiary and quaternary structure of proteins. Cysteine, homocysteine (HCY), and other amino thiols exist in plasma in reduced, oxidized, and protein-bound forms, interacting with each other through redox pathways. Cysteine is the limiting precursor of the major intracellular antioxidant glutathione. The individuals with lower cysteine levels are more prone to damage from reactive oxygen species, which are generally removed either by thiols or by glutathione-linked enzymes. An elevated level of total cysteine also predicts adverse outcomes such as cardiovascular diseases and metabolic syndromes.

Cysteine Colorimetric Microplate Assay Kit is a sensitive assay for determining cysteine in various samples. Cysteine concentration is determined by sodium tungstate dihydrate. The reaction products can be measured at a colorimetric readout at 600 nm.

II. KIT COMPONENTS

Component	Volume	Storage
96-Well Microplate	1 plate	
Assay Buffer	30 mlx 4	4 °C
Reaction Buffer	8 mlx 1	4 °C
Dye Reagent	Powder x 1	4 °C, keep in dark
Dye Reagent Diluent	10 mlx 1	4 °C
Standard	Powder x 1	4 °C
Technical Manual	1 Manual	

III. MATERIALS REQUIRED BUT NOT PROVIDED

1. Microplate reader to read absorbance at 600 nm
2. Distilled water
3. Pipettor, multi-channel pipettor
4. Pipette tips
5. Mortar
6. Centrifuge
7. Timer
8. Ice

IV. REAGENT PREPARATION

Standard: Briefly centrifuge prior to opening. Dissolve in 1 ml distilled water to generate 2 mol/L of standard top solution. Perform 2-fold serial dilutions of the top standard solution using distilled water to make the standard curve. The concentration of standard curve could be 2000/1000/500/250/125/62.5/31.2 mmol/L. Prepare immediately before use.

Dye Reagent: Add 10 ml Dye Reagent Diluent to dissolve before use, then put it in boiling water for 10 minutes. Keep in dark and store at 4 °C for 1 month or -20 °C for 3-4 months after reconstitution.

Note: Divide into small aliquots to avoid repeated freeze-thaw cycles.

V. SAMPLE PREPARATION

1. For cell and bacteria samples

Collect cell or bacteria into centrifuge tube, discard the supernatant after centrifugation, add 1 ml Assay Buffer for 5×10^6 cell or bacteria, sonicate (with power 20%, sonicate 3s, interval 10s, repeat 30 times); centrifuged at 10,000g 4°C for 10 minutes, take the supernatant into a new centrifuge tube and keep it on ice for detection.

2. For tissue samples

Weigh out 0.1 g tissue, homogenize with 1 ml Assay Buffer on ice, centrifuged at 10,000g 4°C for 10 minutes, take the supernatant into a new centrifuge tube and keep it on ice for detection.

3. For liquid samples

Add 0.9 ml Assay Buffer into 0.1 ml liquid sample, centrifuged at 10,000g 4°C for 10 minutes, take the supernatant into a new centrifuge tube and keep it on ice for detection.

VI. ASSAY PROCEDURE

Add following reagents into the microplate:

Reagent*	Sample**	Standard	Blank
Reaction Buffer	80 μ l	80 μ l	80 μ l
Sample	20 μ l	--	
Standard	--	20 μ l	--
Distilled water	--	--	20 μ l
Dye Reagent	100 μ l	100 μ l	100 μ l
Mix, incubate at room temperature for 15 minutes, measured at 600 nm and record the absorbance.			

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.

VI. CALCULATION

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

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

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 protein concentration of sample

$$C = \frac{(C_{\text{Standard}} \times V_{\text{Standard}}) \times (OD_{\text{Sample}} - OD_{\text{Blank}})}{(OD_{\text{Standard}} - OD_{\text{Blank}}) \times C_{\text{Protein}} \times V_{\text{Sample}}} (\mu\text{mol/mg})$$

2.2 According to the quantity of cells or bacteria

$$C = \frac{(C_{\text{Standard}} \times V_{\text{Standard}}) \times (OD_{\text{Sample}} - OD_{\text{Blank}})}{(OD_{\text{Standard}} - OD_{\text{Blank}}) \times N \times (V_{\text{Sample}} / V_{\text{Assay}})} (\mu\text{mol}/10^4)$$

2.3 According to the weight of sample

$$C = \frac{(C_{\text{Standard}} \times V_{\text{Standard}}) \times (OD_{\text{Sample}} - OD_{\text{Blank}})}{(OD_{\text{Standard}} - OD_{\text{Blank}}) \times W \times (V_{\text{Sample}} / V_{\text{Assay}})} (\mu\text{mol/g})$$

2.4 According to the volume of sample

$$C = \frac{(C_{\text{Standard}} \times V_{\text{Standard}}) \times (OD_{\text{Sample}} - OD_{\text{Blank}})}{(OD_{\text{Standard}} - OD_{\text{Blank}}) \times V_{\text{Sample}}} \times 10 (\mu\text{mol/ml})$$

Slope: the absorbance slope of standard curve

n: the dilution factor

C_{Standard} : the standard concentration, mmol/L = $\mu\text{mol/ml}$

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

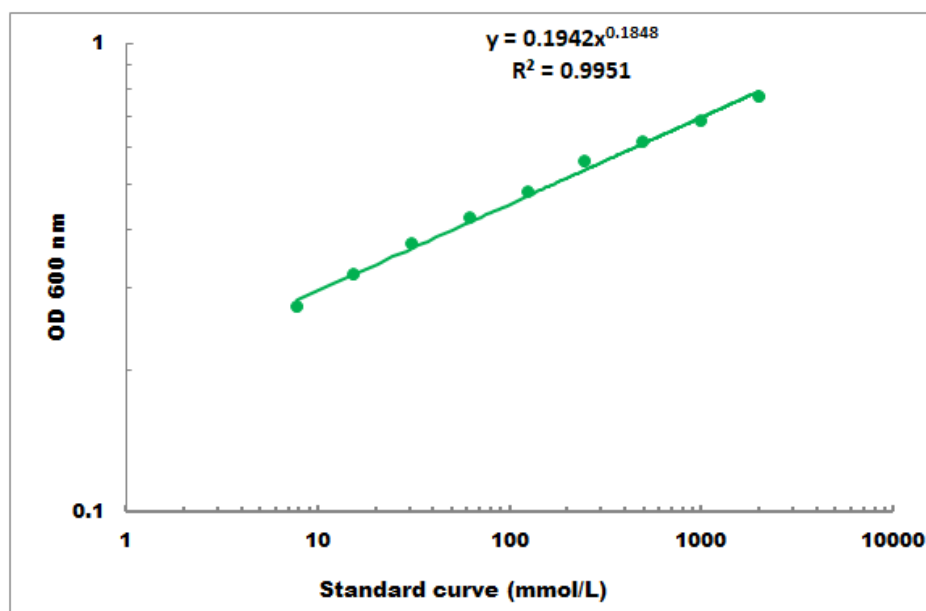
C_{Protein} : the sample protein concentration, mg/ml

W: the weight of sample, g

N: the quantity of cell or bacteria, 10^4

VIII. TYPICAL DATA

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



Detection Range: 20 mmol/L - 2000 mmol/L