



Glutathione Colorimetric Microplate Assay Kit User Manual

Catalog # CAK1006

(Version 1.4D)

Detection and Quantification of Glutathione (GSH) Content in Urine,
Serum, Plasma, 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

Glutathione is a tripeptide of glycine, glutamic acid and cysteine. In the red blood cell, the reduced form of glutathione is vital in maintaining hemoglobin in a reduced state and hence protecting the cells from oxidative damage. Glutathione is involved in detoxification of hydrogen peroxide through glutathione oxidase. Low levels of glutathione are found in deficiencies of key enzymes involved in glutathione metabolism, such as glucose-6-phosphate dehydrogenase, glutathione synthase and glutathione reductase.

Glutathione Colorimetric Microplate Assay Kit provides a convenient tool for detection of reduced glutathione in a variety of samples. The dye reagent can react with reduced glutathione to form a yellow product. The rate of change in the optical density, measured at 412 nm, is directly proportional to glutathione concentration in the sample.

II. KIT COMPONENTS

Component	Volume	Storage
96-Well Microplate	1 plate	
Assay Buffer	30 ml x 4	4 °C
Reaction Buffer	8 ml x 1	4 °C
Dye Reagent	Powder x 1	4 °C, keep in dark
Diluent	4 ml x 1	4 °C
Standard	Powder x 1	4 °C, keep in dark
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. Ice
7. Centrifuge
8. Timer

IV. REAGENT PREPARATION

Standard: Briefly centrifuge prior to opening. Dissolve in 0.65 ml distilled water to generate 10 mmol/L of standard stock solution. Store at -20°C for 2-3 months after reconstitution. Then dilute to 0.5 mmol/L standard top solution by adding 50 µl stock solution into 950 µl distilled water. 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 0.5/0.25/0.125/0.0625/0.0312/0.0156 mmol/L.

Dye Reagent: Add 4 ml Diluent to dissolve before use. Keep in dark and store at 4 °C for 2-3 weeks 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 8000g 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 8000g 4°C for 10 minutes, take the supernatant into a new centrifuge tube and keep it on ice for detection.

3. For serum or plasma samples

Detect directly.

VI. ASSAY PROCEDURE

Warm all reagents to 37°C before use.

Add following reagents into the microplate:

Reagent*	Blank	Standard	Sample**
Reaction Buffer	80 µl	80 µl	80 µl
Sample	--	--	80 µl
Standard	--	80 µl	--
Distilled water	80 µl	--	--
Dye Reagent	40 µl	40 µl	40 µl
Mix, wait for 10 minutes, 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. Calculate the sample concentration in ASSAY PROCEDURE according to the slope of the standard curve

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

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

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

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

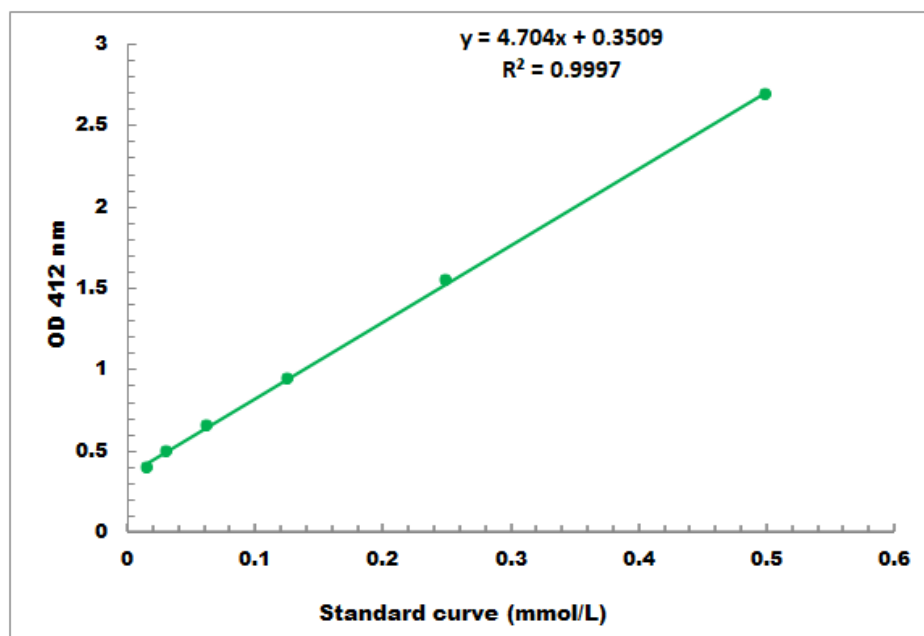
C_{Protein}: the sample protein concentration, mg/ml

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: 0.01mmol/L -0.5mmol/L