Glutathione Microplate Assay Kit

User Manual

Catalog # CAK1006

(Version 1.2D)

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.

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.

The enzyme catalysed 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	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	

Note:

Standard: add 0.65 ml distilled water into the tube, mix; then add 0.05 ml solution into 0.95 ml distilled water, mix. The concentration of the standard will be 0.5 mmol/L. Store at 4°C.

Dye Reagent: add 4 ml Diluent to dissolve before use.

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. 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.

V. ASSAY PROCEDURE

Warm all reagents to 37 °C before use.

Add following reagents into the microplate:

Reagent	Blank	Standard	Sample
Sample	--	--	80 µl
Standard	--	80 µl	--
Distilled water	80 µl	--	--
Reaction Buffer	80 µl	80 µl	80 µl
Dye Reagent	40 µl	40 µl	40 µl
Mix, wait for 10 minutes, 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.

3) Reagents must be added step by step, can not be mixed and added together.

VI. CALCULATION

1. According to the protein concentration of sample

$$\text{Glutathione (μmol/mg)} = (C_{Standard} \times V_{Standard}) \times (OD_{Sample} - OD_{Blank}) / (OD_{Standard} - OD_{Blank}) / (V_{Sample} \times C_{Protein})$$

$$= 0.5 \times (OD_{Sample} - OD_{Blank}) / (OD_{Standard} - OD_{Blank}) / C_{Protein}$$

2. According to the weight of sample

$$\text{Glutathione (μmol/g)} = (C_{Standard} \times V_{Standard}) \times (OD_{Sample} - OD_{Blank}) / (OD_{Standard} - OD_{Blank}) / (V_{Sample} \times W / V_{Assay})$$

$$= 0.5 \times (OD_{Sample} - OD_{Blank}) / (OD_{Standard} - OD_{Blank}) / W$$

3. According to the quantity of cell or bacteria

$$\text{Glutathione (μmol/}10^4\text{ cell)} = (C_{Standard} \times V_{Standard}) \times (OD_{Sample} - OD_{Blank}) / (OD_{Standard} - OD_{Blank}) / (N \times V_{Sample} / V_{Assay})$$

$$= 0.5 \times (OD_{Sample} - OD_{Blank}) / (OD_{Standard} - OD_{Blank}) / N$$

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

W: the weight of sample, g;

$C_{Standard}$: the standard concentration, 0.5 mmol/L = 0.5 μmol/ml;

V_{Sample}: the volume of sample, 0.08 ml;

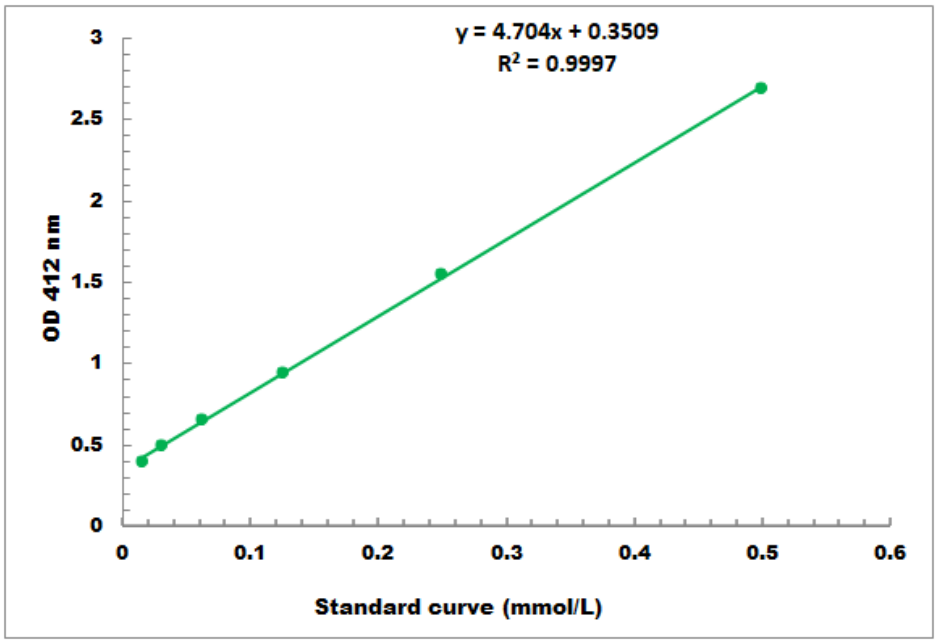
$V_{Standard}$: the volume of standard, 0.08 ml;

V_{Assay}: the volume of Assay buffer, 1 ml;

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

VII. TYPICAL DATA

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



Detection Range: 0.01 mmol/L -0.5 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