GSH/GSSG

Colorimetric Microplate Assay Kit

User Manual

Catalog # CAK1325

(Version 1.1A)

Detection and Quantification of Total, Oxidized and Reduced Glutathione in Blood, Tissue extracts, Cell lysate and Other biological fluids Samples.

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

I. INTRODUCTION

Glutathione exists in cells in two forms - the reduced form (GSH) and the oxidized form (GSSG). The reduced form (GSH), a tripeptide of glycine, glutamic acid and cysteine, is primary biologically active form. GSH donates electrons to neutralize free radicals and repair oxidative damage, during which it itself is oxidized to GSSG. This cycle is essential for maintaining the cellular redox balance and hence protecting the cells from oxidative damage.

GSH/GSSG Colorimetric Microplate Assay Kit provides a convenient tool for detection of GSH, GSSG, and total glutathione separately in a variety of samples. The dye reagent can react with GSH to form a yellow product. The rate of change at 412 nm is directly proportional to glutathione concentration. The glutathione reductase catalyzes the reduction of GSSG to GSH, so total glutathione (GSH + GSSG) can be determined. To detect GSSG specifically, a GSH Quencher is added to remove GSH, preventing reaction with dye reagent.

II. KIT COMPONENTS

Component	Volume	Storage
96-Well Microplate	1 plate	
GSH Quencher	Powder x 1	4 °C, keep in dark
GSH Quencher Diluent A	0.4 ml x 1	4 °C, keep in dark
GSH Quencher Diluent B	11 ml x 1	4 °C
Assay Buffer	Powder x 1	4 °C
Reaction Buffer	30 ml x 2	4 °C
Coenzyme	Powder x 1	-20 °C, keep in dark
Dye Reagent	Powder x 1	4 °C, keep in dark
Dye Reagent Diluent	4 ml x 1	4 °C
Enzyme	Powder x 2	-20 °C, keep in dark
Enzyme Diluent	5 ml x 1	4 °C
Standard	Powder x 1	4 °C, keep in dark
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III. MATERIALS REQUIRED BUT NOT PROVIDED

1. Microplate reader to read absorbance at 412nm
2. Cold PBS
3. Distilled water
4. Pipettor, multi-channel pipettor
5. Pipette tips
6. Mortar
7. Centrifuge
8. Timer

IV. REAGENT PREPARATION

GSH Quencher: Briefly centrifuge prior to opening. Add 0.4 ml GSH Quencher Diluent A to dissolve thoroughly to generate 1M GSH Quencher stock solution, keep in sealed and store at -20 °C for 2 months. Dilute the stock solution to 32 mM GSH inhibitor working solution by GSH Quencher Diluent B before use (eg. 10 µl to 303 µl GSH Quencher Diluent B). Keep working solution at 4 °C and use within 24 hours.

Assay Buffer: Add 15 ml distilled water to dissolve before use, store at -20 °C after reconstitution.

Coenzyme: Add 5 ml Reaction Buffer to dissolve before use, store at 4 °C for 1 month after reconstitution.

Dye Reagent: Add 4 ml Dye Reagent Diluent to dissolve before use, mix, store at 4 °C for 1 month after reconstitution.

Enzyme: Briefly centrifuge prior to opening. Add 1.2 ml Enzyme diluent to each vial to dissolve before use, store at -80 °C for 1 month after reconstitution.

Standard: Briefly centrifuge prior to opening. Add 0.65 ml Reaction Buffer to dissolve to generate Standard stock solution and store at -20 °C for 2 weeks after reconstitution. Then add 0.01 ml into 0.99 ml Reaction Buffer, mix, the concentration will be 100 µmol/L as the top standard solution. Perform serial dilutions using Reaction Buffer to make the standard curve. The concentration of standard curve could be 10/25/50/75/100 µmol/L.

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

V. SAMPLE PREPARATION

For each sample, both GSH-quenched and non-quenched treatments must be performed in parallel.

1. For Blood sample

a. For GSH-quenched: mix 100 µL whole blood with 10 µL GSH inhibitor working solution and freeze at -70 °C. Incubate both samples at RT for 2-10 min.

b. For GSH non-quenched: freeze 100 µL whole blood at -70 °C. Incubate both samples at RT for 2-10 min.

c. Add 65 µL Assay Buffer to 25 µL GSH-quenched or non-quenched samples, briefly vortex to mix and then centrifuge at 14000 rpm for 5 min. Transfer 10 µL of clear supernatant to a clean tube and mix with 222 µL Reaction Buffer.

2. For tissue samples

a. For GSH-quenched: Weigh out 100 mg tissue, homogenize with 1 ml cold PBS and 100 µL GSH inhibitor working solution on ice, centrifuged at 8000g 4 °C for 10 minutes.

b. For GSH non-quenched: Weigh out 100 mg tissue, homogenize with 1 ml cold PBS on ice, centrifuged at 8000g 4 °C for 10 minutes.

c. Take GSH-quenched or non-quenched supernatant into separate centrifuge tubes and keep it on ice. Add 65 µL Assay Buffer to 25 µL supernatant, briefly vortex to mix and then centrifuge at 14000 rpm for 5 min. Transfer 10 µL of clear supernatant to a clean tube and mix with 222 µL Reaction Buffer.

VI. ASSAY PROCEDURE

Add following reagents into the microplate:

Reagent*	GSH-quenched Sample**	Non-quenched Sample**	Standard	Blank
Reaction Buffer	--	--	--	155 µl
Sample	155 µl	155 µl	--	--
Standard	--	--	155 µl	--
Coenzyme	20 µl	20 µl	20 µl	20 µl
Enzyme	10 µl	10 µl	10 µl	10 µl
Dye Reagent	15 µl	15 µl	15 µl	15 µl
Mix, measured at 412 nm immediately 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.

VII. CALCULATION

1. Subtract OD of Blank from OD of all standards. Plot the ΔOD values against standard concentrations. Determine the confident(a) and power(b) using "Power" regression fitting. The standard curve is $y=ax^b$.

2.Calculate the GSH, GSSG, and total glutathione concentration of blood samples according to the standard curve

$$C_{Total} = \sqrt[b]{\frac{OD_{No\text{-}quenched\ Sample} - OD_{Blank}}{a}} \times n \times 0.001 \quad (mmol/L)$$

$$C_{GSSG} = \sqrt[b]{\frac{OD_{GSH\text{-}quenched\ Sample} - OD_{Blank}}{a}} \times 0.5 \times n \times 0.001 \quad (mmol/L)$$

$$C_{GSH} = C_{Total} - 2 \times C_{GSSG} \quad (mmol/L)$$

3.Calculate the GSH, GSSG, and total glutathione concentration of tissue samples according to the standard curve

$$C_{Total} = \sqrt[b]{\frac{OD_{No\text{-}quenched\ Sample} - OD_{Blank}}{a}} \times n \times 0.001 \times V_{PBS} \times {}^{1}/_{W} \quad (mmol/mg)$$

$$C_{GSSG} = \sqrt[b]{\frac{OD_{GSH\text{-}quenched\ Sample} - OD_{Blank}}{a}} \times 0.5 \times n \times 0.001 \times V_{PBS} \times {}^{1}/_{W} \quad (mmol/mg)$$

$$C_{GSH} = C_{Total} - 2 \times C_{GSSG} \quad (mmol/mg)$$

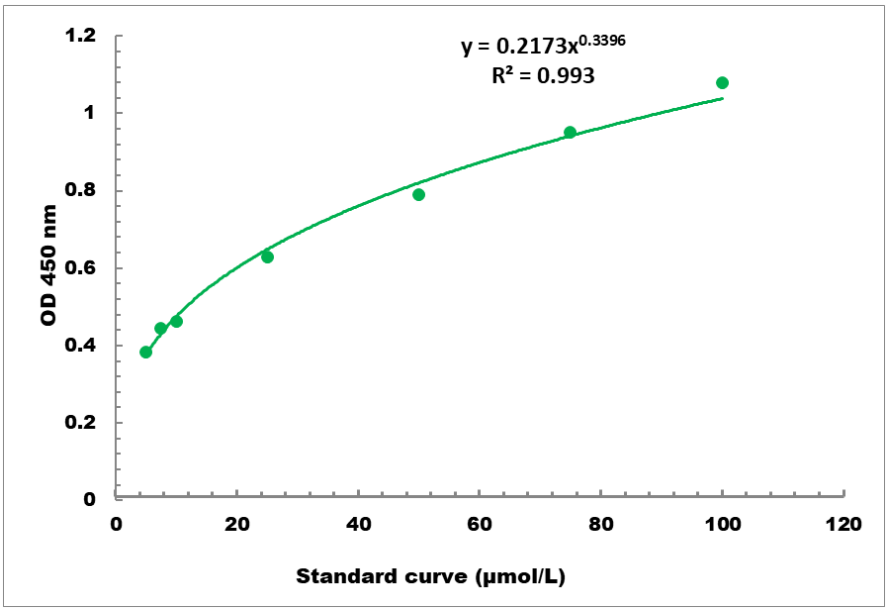
n: the dilution factor, n=80

V_{PBS}: the volume of PBS, V_{PBS} = 1 ml

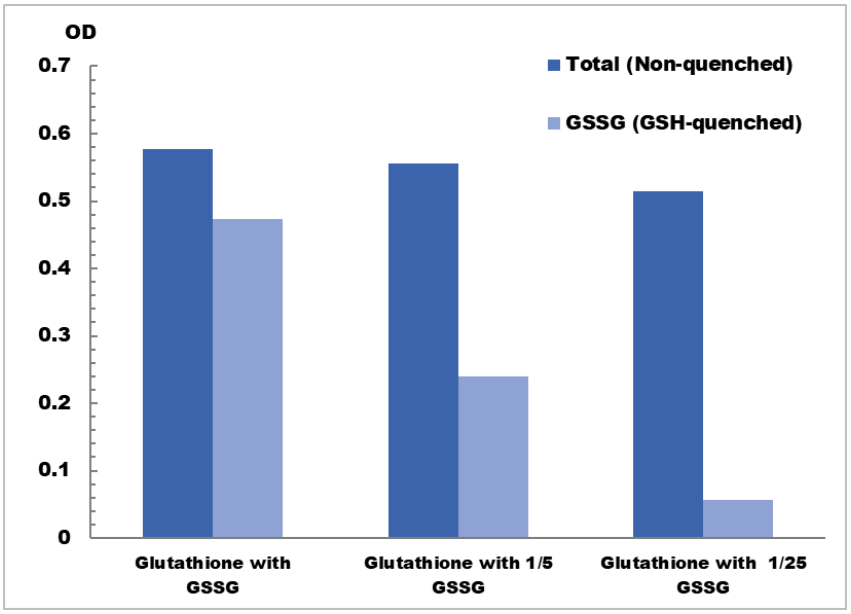
W: the weight of sample, W = 100 mg

VIII. TYPICAL DATA

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



Detection Range: 5 µmol/L - 100 µmol/L



Positive Control reaction with decreasing the concentration of GSSG