GSH/GSSG Ratio Assay Kit (Fluorometric)

Introduction

Glutathione is a tripeptide composed of glutamic acid (Glu), cysteine (Cys), and glycine (Gly). Glutathione is mainly found in cells in two forms, namely reduced glutathione (GSH) and oxidized glutathione disulfide (GSSG). Reduced glutathione is highly reducible and is the main active form in cells (accounting for >90%). Oxidized glutathione is formed by the dehydrogenation of two GSH. When the level of oxidative stress in cells increases, the content of oxidized glutathione rises, causing the GSH/GSSG ratio to decrease. Thus, the GSH/GSSG ratio serves as a key indicator of cellular oxidative stress.

This kit uses Thiolite Green to quantify the GSH/GSSG ratio. Thiolite Green can react with GSH and produce an intense green fluorescence (Ex/Em=504/522 nm) to quantify the GSH content in the sample. At the same time, this kit provides a GSSG Probe, which can reduce GSSG to GSH to detect the total GSH content. The GSH/GSSG ratio can be calculated after obtaining GSH and Total GSH content. For detecting GSH only, this kit is enough to be used for 200 assays.

Components and Storage

Components \ Size	200 Assays	Storage
Thiolite Green 100X	100 µL	-20°C away from light
DMSO	500 µL	-20°C
Assay Buffer	30 mL	-20°C
GSH	1 vial	-20°C
GSSG	1 vial	-20°C
GSSG Probe A	10 µL	4°C away from light
GSSG Probe B	1 vial	4°C away from light
Shipping: Blue ice	**Shelf life: 1 year**	

Protocol

1. Materials required but not supplied

    1) Lysis buffer (5% NP-40 in PBS, pH 6.5)

    2) Trichloroacetic acid (TCA)

3) Sodium bicarbonate ($NaHCO_3$)

2. Reagent preparation

1) Dissolve the GSH vial in 3.2 mL of Assay Buffer to make a 1 mM GSH standard stock solution. Keep the stock solution on ice when using. Aliquot and store unused stock solution at -20°C to avoid repeated freeze-thaw cycles.

2) Dissolve the GSSG vial in 1.63 mL of ddH_2O to make a 1 mM GSSG standard stock solution. Keep the stock solution on ice when using. Aliquot and store unused stock solution at -20°C to avoid repeated freeze-thaw cycles.

3) Dilute an appropriate amount of Thiolite Green 100X in DMSO to make a Thiolite Green 25X. Keep the Thiolite Green 25X on ice when using. Aliquot and store Thiolite Green 100X at -20°C to avoid repeated freeze-thaw cycles.

4) Dissolve GSSG Probe B vial in 100 µL of sterile water to make the GSSG Probe B solution. Keep the solution on ice when using. Aliquot and store unused solution at -80°C to avoid repeated freeze-thaw cycles, stable for 1 month.

5) To make the GSH working solution, add 500 µL of Assay Buffer per 400 µL of Thiolite Green 25X, continue to add Assay Buffer to a total volume of 5 mL, and finally supplement the volume with Assay Buffer to 10 mL. The volume of GSH working solution can be adjusted according to the experiment. **The working solution should be used within 1-2 hours after preparation. Therefore, it is better to prepare it when samples and standards preparation are ready.**

***Note:** Thiolite Green is a highly hydrophobic probe, and slight adhesion to the vessel wall is a normal phenomenon. However, excessive adhesion may reduce detection sensitivity.

6) Mix 10 µL of GSSG Probe A and 100 µL of GSSG Probe B solution, then add 5 mL of GSH Working Solution, and vortex to make the Total GSH Working Solution. **The working solution should be used within 1-2 hours after preparation. Therefore, it is better to prepare it when samples and standards preparation are ready.**

3. GSH and GSSG standards preparation

1) Add 990 µL of Assay Buffer per 10 µL of 1 mM GSH to make a 10 µM GSH standard. Dilute 200 µL of 10 µM GSH standard in Assay Buffer to prepare 5 µM, 2.5 µM, 1.25 µM, 0.625 µM, 0.3125 µM and 0.1563 µM GSH standards.

2) Add 990 µL of Assay Buffer per 10 µL of 1 mM GSSG to make a 10 µM GSSG standard. Dilute 200 µL of 10 µM GSSG standard in Assay Buffer to prepare 5 µM, 2.5 µM, 1.25 µM, 0.625 µM, 0.3125 µM, 0.1563 µM, and 0.0781 µM GSSG standards.

***Note:** The diluted GSH and GSSG standards are unstable and are recommended to be used within 4 h after preparation.

4. Sample preparation

A. For cell samples

1) Use fresh samples as much as possible. Harvest cells (initial recommendation = 0.1-1 x 10^8 cells) and wash once with pre-chilled PBS.

2) Resuspend cells in 100 µL of lysis buffer (0.5% NP-40 in PBS, pH 6.5). Pipet up and down several times to homogenize cells.

3) Centrifuge at 4°C, 12000 x g for 15 min.

4) Transfer supernatant to a new tube and place on ice.

5) Perform step 5 for deproteinization.

B. For tissue samples

1) Weigh an appropriate amount of tissue (initial recommendation = 20 mg) and wash once with pre-chilled PBS.

2) Add 400 µL of lysis buffer (0.5% NP-40 in PBS, pH 6.5) per 20 mg of tissue and homogenize on ice.

3) Centrifuge at 4°C, 12000 x g for 15 min.

4) Transfer supernatant to a new tube and place on ice.

5) Perform step 5 for deproteinization.

C. For plasma, serum, and other liquid samples

1) Use fresh samples for deproteinization in step 5.

5. Deproteinization

Tissue, cell, and biological fluid samples may contain proteins that interfere with detection, so samples need to be deproteinized. Here use TCA/$NaHCO_3$ method for deproteinization.

1) Add 5 volumes pre-cooled 50% TCA into 1 volume of sample supernatant and vortex briefly to mix well.

2) Incubate the samples on ice for 5-10 min.

3) Centrifuge at 4°C, 12000 x g for 5 min.

4) Transfer supernatant to a new tube.

5) Then drop $NaHCO_3$ solution into the supernatant until the solution pH is 4-6. Use pH paper to test 1 µL of the sample.

***Note:** Try to avoid pH > 7 after neutralization, as GSH is unstable and easily oxidized at pH >7.

6) Centrifuge at 4°C, 13000 x g for 15 min.

7) Transfer supernatant to a new tube and place on ice. The samples are ready for testing.

6. GSH and Total GSH assay

1) Refer to the table below to transfer blank controls (BL, Assay Buffer), samples (TS), GSH standard dilutions (0.1563-10 μM), and GSSG standard dilutions (0.0781-5 μM) into 96-well plates, requiring 50 μL per well. A black 96-well plate is recommended.

Group A: Detection of GSH				Group A: Detection of Total GSH			
BL	BL	TS	TS	BL	BL	TS	TS
GSH1	GSH1	…	…	GSSG1	GSSG1	…	…
GSH2	GSH2	…	…	GSSG2	GSSG2	…	…
GSH3	GSH3	…	…	GSSG3	GSSG3	…	…
GSH4	GSH4	…	…	GSSG4	GSSG4	…	…
GSH5	GSH5	…	…	GSSG5	GSSG5	…	…
GSH6	GSH6	…	…	GSSG6	GSSG6	…	…
GSH7	GSH7	…	…	GSSG7	GSSG7	…	…

2) For group A, add 50 μL of GSH working solution per well. For group B, add 50 μL of Total GSH working solution per well. Incubate at room temperature in the dark for 60 min.

***Note:** The optimal incubation time can be adjusted within 10-60 minutes depending on the experiment.

3) Measure fluorescence signal at Ex/Em=504/522 nm.

7. Analysis

1) Subtract the group A background (BL) from all group A sample and standards readings. Subtract the group B background (BL) from all group B sample and standards readings.

2) Establish standard curves with the corrected values.

3) Calculate the GSH concentration in the sample by applying the group A corrected sample readings to the standard curve of GSH. Include the sample dilution factor in calculations.

4) Calculate the Total GSH concentration in the sample by applying the group B corrected sample readings to the standard curve of GSSG. Include the sample dilution factor in calculations.

5) Because 1 mole of GSSG can be converted to 2 moles of GSH, GSSG = (Total GSH-GSH)/2

Note

1. If sample readings fall outside the standard curve range, dilute or concentrate the sample accordingly and repeat the measurement.

2. It is recommended to use fresh samples every time. If you cannot detect in time, it is recommended to store samples after completing the sample preparation and deproteinization.

3. For your safety and health, please wear lab coats and gloves during the experiment.

4. For research use only. Not to be used in clinical diagnostic or clinical trials.

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