Amplite™ Rapid Fluorimetric Glutathione GSH/GSSG Ratio Assay Kit *Green Fluorescence*

Catalog number: 10060
Unit size: 200 Tests

Component	Storage	Amount
Component A: Thiolite™ Green 520WS	Freeze (< -15 °C), Minimize light exposure	1 vial
Component B: Assay Buffer	Freeze (< -15 °C)	1 bottle (25 mL)
Component C: GSH Standard	Freeze (< -15 °C)	1 vial (62 µg)
Component D: GSSG Probe	Freeze (< -15 °C), Minimize light exposure	1 bottle (lyophilized powder)
Component E: GSSG Standard	Freeze (< -15 °C), Minimize light exposure	1 vial (124 µg)

OVERVIEW

When cells are exposed to increased levels of oxidative stress, GSSG will accumulate and the ratio of GSH to GSSG will decrease. The glutathione redutase recycles GSSG to GSH with simultaneous oxidation of b-nicotinamide adenine dinuclecotide phosphate. The monitoring of GSH/GSSG ratio and the quantification of GSSG in biological samples are essential for evaluating the redox and detoxification status of cells and tissues in relation to the protective role of glutathione against oxidative and free-radical-mediated cell injury. There are a few reagents or assay kits available for the quantitation of thiols in biological systems. However, all the commercial kits either lack sensitivity or have tedious protocols. Our Amplite™ Rapid Fluorimetric GSH/GSSG Ratio Kit (#10060) provides an ultrasensitive assay to quantitate GSH in the sample. The kit uses a proprietary water-soluble non-fluorescent dye that becomes strongly fluorescent upon reacting with thiol. The kit provides a sensitive, one-step fluorimetric method to detect as little as 1 picomole of cysteine or GSH in a 100 µL assay volume. The assay can be performed in a convenient 96-well or 384-well microtiter-plate format and easily adapted to automation without a separation step. Its signal can be easily read by a fluorescence microplate reader.

AT A GLANCE

Protocol Summary

1. Prepare GSH working solution (50 µL)
2. Add GSH standards and/or GSSG standards or test samples (50 µL)
3. Incubate at RT for 10 to 60 minutes
4. Monitor the fluorescence increase at Ex/Em = 490/525 nm (Cutoff = 515 nm)

Important Thaw all the kit components at room temperature before starting the experiment.

KEY PARAMETERS

Fluorescence microplate reader

Excitation	490 nm
Emission	525 nm
Cutoff	515 nm
Recommended plate	Solid black

PREPARATION OF STOCK SOLUTIONS

Unless otherwise noted, all unused stock solutions should be divided into single-use aliquots and stored at -20 °C after preparation. Avoid repeated freeze-thaw cycles.

1. GSH standard solution (1 mM)
Add 200 µL of Assay Buffer (Component B) into the vial of GSH Standard (Component C) to make 1 mM (1 nmol/µL) GSH standard solution.

2. GSSG standard stock solution (1 mM)
Add 200 µL of ddH_2O into the vial of GSSG Standard (Component E) to make 1 mM (1 nmol/µL) GSSG standard solution.

3. Thiolite™ Green 520WS stock solution (100X)
Add 100 µL of ddH_2O into the vial of Thiolite™ Green 520WS (Component A) to make 100X Thiolite™ Green 520WS stock solution.

Note Avoid light.

PREPARATION OF STANDARD SOLUTION

For convenience, use the Serial Dilution Planner:
https://www.aatbio.com/tools/serial-dilution/10060

GSH or GSSG standard
Prepared serially diluted GSH standards (0 to 10 µM):Add 10 µL of 1 mM (1 nmol/µL) GSH standard solution to 990 µL of Assay Buffer (Component B) to generate 10 µM (10 pmol/µL) GSH standard solution (GSH7). Take 10 µM (10 pmol/µL) GSH standard solution (GSH7) and perform 1:2 serial dilutions in Assay Buffer (Component B) to get serially diluted GSH standards (GSH6 - GSH1). Note: Diluted GSH standard solution is unstable. Use within 4 hours. Prepare serially diluted GSSG standards (0 to 5 µM):Add 10 µL of 1 mM (1 nmol/µL) GSSG standard solution to 990 µL of Assay Buffer (Component B) to generate 10 µM (10 pmol/µL) GSSG standard solution. Take 10 µM (10 pmol/µL) GSSG standard solution and perform 1:2 serial dilutions in Assay Buffer (Component B) to get serially diluted GSSG standards (GSSG7 - GSSG1). Note: Diluted GSSG standard solution is unstable. Use within 4 hours.

PREPARATION OF WORKING SOLUTION

1. GSH working solution (GSH-WS)
Add 100 µL of 100X Thiolite™ Green 520WS stock solution into 10 mL of Assay Buffer (Component B) and mix well by vortexing.

Note This GSH working solution (GSH-WS) is enough for two 96-well plates. It is stable at 4 °C for at least 4 hours when protected from light.

2. Total GSH working solution (TGSH-WS)
Add 5 mL of GSH-WS into the bottle of GSSG Probe (Component D) and mix them well.

Note This Total GSH working solution (TGSH-WS) is enough for one 96-well plates. It is unstable at room temperature, and should be used promptly within 2 hours.

Note Avoid exposure to light.

Note Alternatively, one can make a 25X GSSG Probe by adding 200 µL of ddH_2O into the bottle of GSSG Probe (Component D), and then prepare the TSGS-WS by mix the stock solution with GSH-WS proportionally.

SAMPLE EXPERIMENTAL PROTOCOL

Table 1. Layout of GSH standards, GSSG standards, and test samples in a solid black 96-well microplate. GSH= GSH Standards (GSH1 - GSH7, 0.15 to 10 µM), GSSG= GSSG Standards (GSSG1 - GSSG7, 0.078 to 5 µM), BL=Blank Control, TS=Test Samples.

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Panel A				Pannel B			
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		

Table 2. Reagent composition for wells.

Note Add test samples into wells in both Panel A and Panel B.

Well	Volume	Reagent
GSH1 - GSH7	50 µL	Serial Dilutions (0.15 to 10 µM)
GSSG1-GSSG7	50 µL	Serial Dilutions (0.078 to 5 µM)
BL	50 µL	Assay Buffer
TS	50 µL	test sample

1. Prepare GSH standards (GSH), blank controls (BL), and test samples (TS) according to the layout provided in Tables 1 and 2. For a 384-well plate, use 25 µL of reagent per well instead of 50 µL. When just GSH assay is needed, fill ONLY the wells in two left columns (Panel A) according to Table 1. When Total GSH assay is needed, fill the wells in both Panel A (left) and Panel B (right) according to Table 1.

   Note Treat cells or tissue samples as desired.

2. Add 50 µL of GSH working solution (GSH-WS) into each well of GSH standards, blank controls, and test samples to make the total GSH assay volume of 100 µL/well. For a 384-well plate, add 25 µL of GSH-WS into each well instead, for a total volume of 50 µL/well.

3. If total GSH (in reduced and oxidized states) assay is needed, prepare Total GSH working solution (TGSH-WS) and GSSG standards. Add GSSG standards (GSSG), blank controls (BL), and test samples (TS) according to the layout provided in Tables 1 and 2. For a 384-well plate, use 25 µL of reagent per well instead of 50 µL. Then add 50 µL of TGSH-WS to each well of GSSG standards, blank controls, and test samples to amke the total assay volume 100 µL/well. For a 384-well plate, add 25 µL of TGSH-WS into each well instead, for a total volume of 50 µL/well.

4. Incubate the reaction at room temperature for 10 to 60 minutes, protected from light.

5. Monitor the fluorescence increase with a fluorescence plate reader at Ex/Em = 490/525 nm (Cutoff = 515 nm).

EXAMPLE DATA ANALYSIS AND FIGURES

The change of fluorescence intensity with GSH concentration can be described as a linear regression: Log(y) = A + B * Log(x) *Note:* The equation is generated by most instrument software. [GSH] can be calculated by the equation from the GSH standard calibration curve.[Total GSH] can be calculated by the equation from the Total GSH standard calibration curve.

GSSG Concentration: [GSSG] = ([Total GSH] - [GSH])/2

GSH/GSSG Ratio Determination: [GSH]/[GSSG]

Note **0.078 to 5 µM GSSG which is equivalent to 0.156 to 10 µM GSH.**

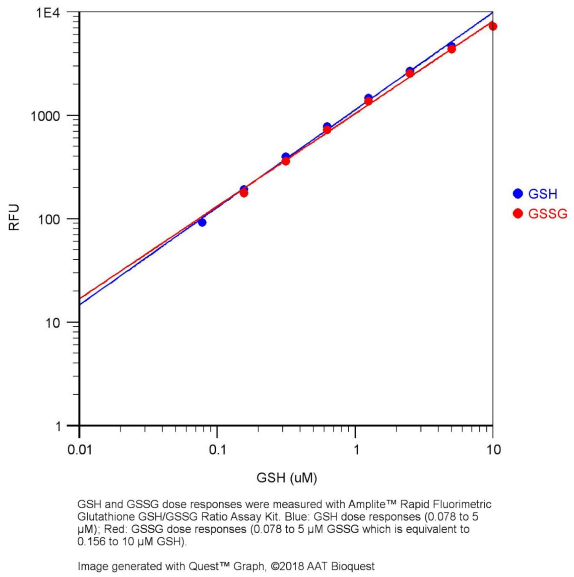

Figure 1. GSH and GSSG dose responses were measured with Amplite™ Rapid Fluorimetric Glutathione GSH/GSSG Ratio Assay Kit. Blue: GSH dose responses (0.078 to 5 µM); Red: GSSG dose responses (0.078 to 5 µM GSSG which is equivalent to 0.156 to 10 µM GSH).

DISCLAIMER

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