Amplite™ Fluorimetric Total Thiol Quantitation Assay Kit *Green Fluorescence*

Catalog number: 5524
Unit size: 200 Tests

Component	Storage	Amount
Component A: Thiolite™ Green	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: DMSO	Freeze (< -15 °C)	1 vial (400 µL)

OVERVIEW

The detection and measurement of free thiol (such as free cysteine, glutathione and cysteine residues in proteins) is one of the essential tasks for investigating biological processes and events in many biological systems. The monitoring of reduced (GSH) and oxidized glutathione in biological samples is 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. Disorders of cysteine metabolism include cystinosis, an autosomal recessive disease produced by a defect in lysosomal transport, and cystinuria, a common heritable disorder of amino acid transport. There are few reagents or assay kits available for quantitating thiols in biological systems. However, all the commercial kits either lack sensitivity or have tedious protocols. Our Amplite™ Fluorimetric Total Thiol Qutitation Kit provides an ultrasensitive fluorimetric assay for quantitating thiols that exist either in a small molecule. The kit uses a proprietary 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 (10 nM in concentration). The assay is rapid and robust. It can be performed in a convenient 96-well or 384-well microtiter-plate format and easily adapted to automation. For rapid quantifying thiol groups in a protein, we recommend you use our kit #5529 that is optimized for quantifying protein thiols.

AT A GLANCE

Protocol Summary

1. Prepare GSH working solution (50 µL)
2. Add GSH 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 ddH_2O into the vial of GSH Standard (Component C) to make 1 mM (1 nmol/µL) GSH standard solution.

2. Thiolite™ Green stock solution (100X)

Add 100 µL of DMSO (Component D) into the vial of Thiolite™ Green (Component A) to make 100X Thiolite™ Green stock solution.

Note Alternatively, if precipitation is observed while making working solution, one can make 50X stock solution using 200 µL DMSO solution.

PREPARATION OF STANDARD SOLUTION

For convenience, use the Serial Dilution Planner:
https://www.aatbio.com/tools/serial-dilution/5524

GSH standard
Add 30 µL of 1 mM (1 nmol/µL) GSH standard solution to 970 µL of Assay Buffer (Component B) to generate 30 µM (30 pmol/µL) GSH standard solution. Take 30 µM (30 pmol/µL) GSH standard solution and perform 1:3 serial dilutions to get serially diluted GSH standards (SD7-SD1) with Assay Buffer (Component B). Note: Diluted GSH standard solution is unstable. Use within 4 hours.

PREPARATION OF WORKING SOLUTION

Add 50 µL of 100X Thiolite™ Green stock solution into 5 mL of Assay Buffer (Component B) and mix well to make GSH working solution.

Note This GSH working solution is enough for one 96-well plate. It is unstable at room temperature, and should be used promptly within 2 hours. Avoid exposure to light.

Note Alternatively, one can make GSH working solution by adding 100X Thiolite™ Green stock solution with Assay Buffer (Component B) proportionally.

Note If precipitation is observed with this protocol, then one can make 50X dilution (See note in stock solution (2)) and use 100 µL of 50X Thiolite™ Green stock solution into 5 mL of Assay Buffer (Component B).

SAMPLE EXPERIMENTAL PROTOCOL

Table 1. Layout of GSH standards and test samples in a solid black 96-well microplate. SD = GSH Standards (SD1 - SD7, 0.014 to 10 µM); BL=Blank Control; TS=Test Samples

BL	BL	TS	TS
SD1	SD1	...	...
SD2	SD2	...	...
SD3	SD3		
SD4	SD4		
SD5	SD5		
SD6	SD6		
SD7	SD7		

Table 2. Reagent composition for each well.

Well	Volume	Reagent
SD1-SD7	50 µL	Serial Dilutions (0.014 to 10 µM)
BL	50 µL	Assay Buffer
TS	50 µL	Test Sample

1. Prepare GSH standards (SD), 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. *Note:* Treat cells or tissue samples as desired.

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

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

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

EXAMPLE DATA ANALYSIS AND FIGURES

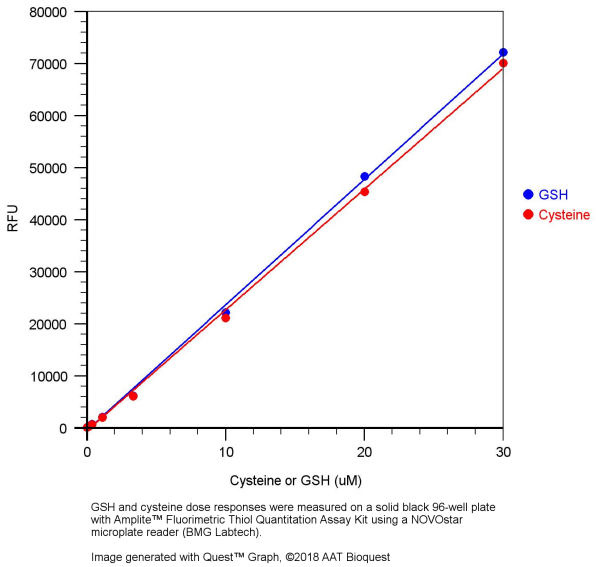

Figure 1. GSH and cysteine dose responses were measured on a solid black 96-well plate with Amplite™ Fluorimetric Thiol Quantitation Assay Kit using a NOVOstar microplate reader (BMG Labtech).

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