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

Catalog number: 5528
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)

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 Thiol Qutitation Kit provides an ultrasensitive fluorimetric assay for quantitating thiols that exist either in a small molecule. 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 (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 recommendyou use our kits #5529 and #5530 that are optimized for quantifying proteinthiols.

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

Instrument:	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 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.

PREPARATION OF STANDARD SOLUTION

GSH standard

For convenience, use the Serial Dilution Planner:
https://www.aatbio.com/tools/serial-dilution/5528

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 520WS 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 stable at 4^0C for 6 hours when kept from light.

Note Alternatively, one can make GSH working solution by adding 100X Thiolite™ Green 520WS stock solution with Assay Buffer (Component B) proportionally.

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		

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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

The reading (RFU) obtained from the blank standard well is used as a negative control. Subtract this value from the other standards' readings to obtain the base-line corrected values. Then, plot the standards' readings to obtain a standard curve and equation. This equation can be used to calculate Cysteine or GSH samples. We recommend using the Online Linear Regression Calculator which can be found at:

https://www.aatbio.com/tools/linear-logarithmic-semi-log-regression-online-calculator

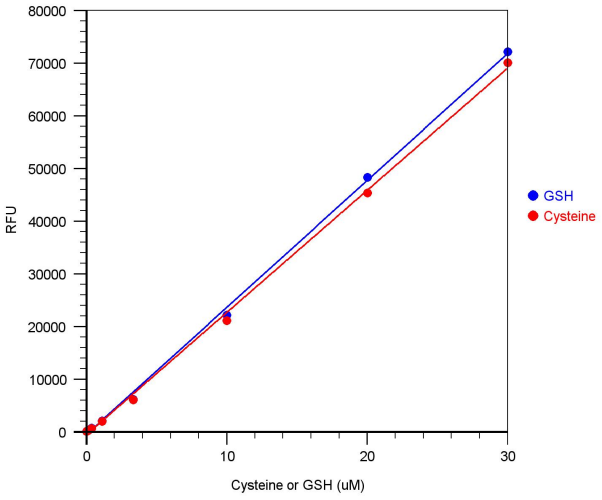

Figure 1. GSH and cysteine dose responses were measured on a solid black 96-well plate with Amplite™ Fluorimetric Thiol Quantitation Assay Kit using a NOVOstar microplate reader (BMG Labtech).

DISCLAIMER