Amplite™ Fluorimetric Thiol Quantitation Kit *Green Fluorescence*

Ordering Information:
Product number: 5524 (200 assays)

Instrument Platform:
Fluorescence microplate readers

Storage Conditions:
Keep in -20°C avoid moisture and light

Introduction

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 thiol of cysteine is a powerful nucleophile, and cysteine is the most easily oxidized of the amino acids, forming covalent crosslinks between peptide chains. Because of the unique reactivity, cysteines in proteins have been the site, both deliberately and inadvertently, of a vast number of modification reactions. These modifications include oxidations with a variety of oxidizing agents, reaction with a-halo acids and organic mercurial derivatives, and the reversible formation of disulfides by reaction with a wide variety of reagents including tetrathionate to produce *S*-sulfocysteine and alkyl or aryl methanethiosulfonates to yield organic disulfides.

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. Cysteine is unique among the amino acids found in proteins.

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 or on a protein. 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; Figure 1). The assay can be performed in a convenient 96-well or 384-well microtiter-plate format and easily adapted to automation with no separation steps required. Its signal can be easily read by fluorescence microplate reader with Ex/Em = 490 nm/520 nm.

Kit Key Features

Broad Application:	Can be used for quantifying thiol and sulfide in a variety of biological systems (e.g., plasma, urine and cell extracts)
Sensitive:	Detect as low as 1 picomole of thiol.
Continuous:	Easily adapted to automation with no separation required.
Convenient:	Formulated to have minimal hands-on time. No wash is required.
Non-Radioactive:	No special requirements for waste treatment.

Kit Components

Components	Amount
Component A: Thiolite Green™	1 vial
Component B: Assay Buffer	1 bottle (25 ml)
Component C: GSH standard	1 vial (62 µg)
Component D: DMSO	1 vial (100 µL)

Assay Protocol for one 96-well plate

Brief Summary

Prepare Thiolite Green™ reaction mixture (50 µL) → Add GSH standards or test samples (50 µL) → Incubate at room temperature for 10 min-1 h → Read fluorescence at Ex 490 nm/Em 520nm nm

Note: Thaw all the kit components to room temperature before starting your experiment.

1. Prepare GSH standard stock solution:

Prepare GSH standard stock solution: Add 200 µL of ddH2O into the GSH standard vial (Component C) to make 1 mM (1 nmol/µL) stock solution.

Note: The unused GSH solution should be divided as single use aliquots and stored at -20°C.

2. Prepare 400X Thiolite Green™ stock solution:

Prepare Thiolite Green™ stock solution: Add 25 µL of DMSO (Component D) into the Thiolite Green vial (Component A) to make 400X stock solution.

Note: The unused Thiolite Green solution should be divided as single use aliquots and stored at -20°C and avoid from light.

3. Prepare GSH reaction mixture:

Prepare the GSH reaction mixture: Add 12.5 µL of 400X Thiolite Green™ stock solution (from step 2) into 5 ml assay buffer (Component B), mixed well.

4. Prepare Serial GSH (0 to 30 µM) solutions

4.1 Add 30 µL of GSH standard stock solution (from step 1) to 970 µl assay buffer (Component B) to generate 30 µM (30 pmol/µL) standard.
Note: Diluted GSH standard solution is unstable, should be used within 4 hours.

4.2 Take 200 µL of 30 µM solution to perform 1:3 serial dilutions to get 10, 3, 1, 0.3, 0.1, 0.03, 0.01 and 0 standard GSH solutions.

4.3 Add GSH standards and GSH-containing or other thiol-containing test samples into a 96-well solid black microplate as described in Tables 1 and 2
Note: Treat your cell or tissue samples as desired.

Table 1. Layout of GSH standards and test samples in a solid black 96-well microplate:

BL	BL	TS	TS	….	….						
GS1	GS1	….	….	….	….						
GS2	GS2										
GS3	GS3										
GS4	GS4										
GS5	GS5										
GS6	GS6										
GS7	GS7										

Note: GS= GSH standards, BL=Blank control, TS=test samples.

Table 2. Reagent composition for each well:

GSH Standard	**Blank Control**	**Test Sample**
Serial dilutions* (50 µL)	Assay buffer: 50 µL	50 µL

**Note: Add the serially diluted GSH standards from 0.01 µM to10 µM into wells from NS1 to NS7 in duplicate.*

5. Run GSH Assay

5.1 Add 50 µL of GSH assay mixture (from step 3) to each well of the GSH standard, blank control, and test samples (see step 4.3) so that the total GSH assay volume is 100 µL/well
Note: For a 384-well plate, add 25 µL sample, 25 µL of GSH reaction mixture *per well.*

4.1 Incubate the reaction for 10 minutes to 1 hour at room temperature, protected from light.

4.2 Monitor the fluorescence increase with 490 nm excitation and 520 nm emission by using a fluorescence plate reader.

6. Run Data Analysis

The fluorescence in blank wells (with the assay buffer only) is used as a control, and is subtracted from the values for those wells with the GSH reactions. The typical data are shown in Figure 1 (GSH standard curve).
Note: The fluorescence background increases with time, thus it is important to subtract the fluorescence intensity value of the blank wells for each data point.

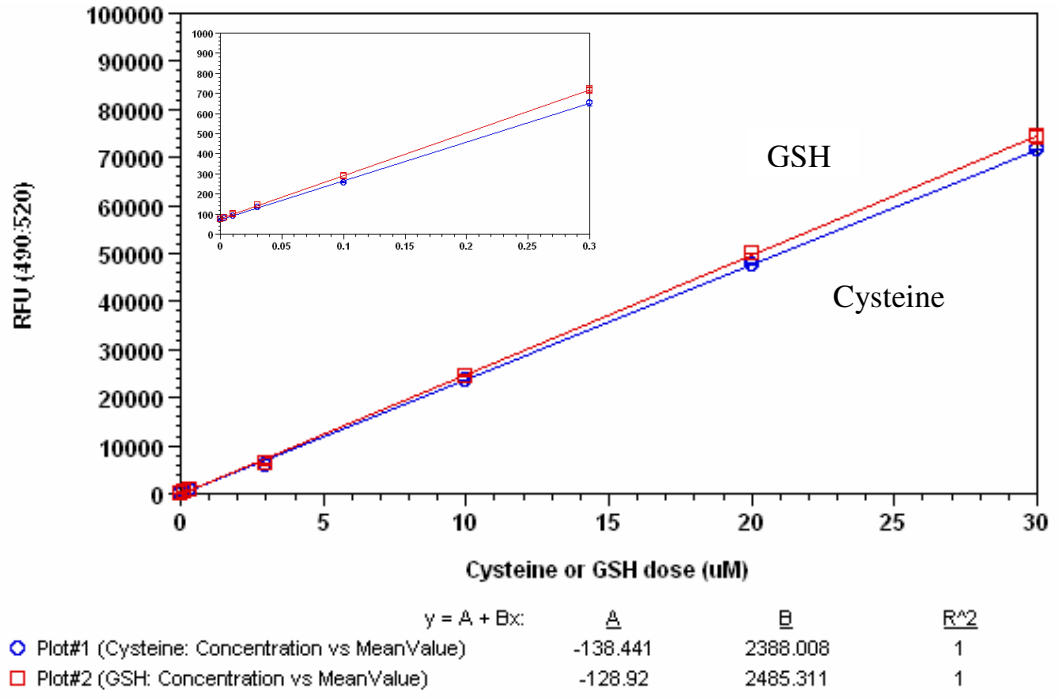

Figure 1. GSH and Cysteine dose response on 96-well black plate was measured with Amplite™ Fluorimetric Thiol Quantitation Assay Kit using a BMG LabTech NOVOStar microplate reader. As low as 10 nM (1 pmol/well) of GSH or Cysteine can be detected with 10 minutes incubation time (n=3). The insert shows the low levels of thiol detection.

References:

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