Amplite™ Fluorimetric Thiol Quantitation Kit

Green Fluorescence

Ordering Information	*Storage Conditions*	*Instrument Platform*
Product Number: 5524 (200 assays)	Keep at -20 °C Avoid exposure to moisture and light	Fluorescence microplate readers

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. There are a few reagents or assay kits available for quantitating thiol content in biological systems. All the commercial kits either lack sensitivity or have tedious protocols.

Our Amplite™ Fluorimetric Thiol Qutitation Assay Kit provides an ultrasensitive fluorimetric assay to quantitate thiol content that exists either in a small molecule or on a protein. The proprietary non-fluorescent dye used in the kit becomes strongly fluorescent upon reacting with thiol. The kit can detect as little as 1 picomole of cysteine or GSH in a 100 µL assay volume (10 nM). 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. The thiol sensor used in the kit generates a strongly fluorescent adduct upon reacting with a thiol compound. The resulted adduct has the spectral proterties almost identical to those of fluorescein. In addition, both absorption and emission spectra of the thiol adduct are pH-independent, making this assay kit highly robust. The signal can be easily read by a fluorescence microplate reader at Ex/Em = 490/520 nm.

Kit Key Features

Broad Application:	Can be used for quantifying thiol 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 without a separation step.
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 (200 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 minutes - 1 hour → Monitor the fluorescence increase at Ex/Em = 490/520 nm

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

1. Prepare GSH standard stock solution:

Add 200 µL of ddH_2O into the GSH standard vial (Component C) to make 1 mM (1 nmol/µL) stock solution.

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

Ordering: sales@aatbio.com; Tel: 800-990-8053 or 408-733-1055; Fax: 408-733-1304
Technical Support: support@aatbio.com; Tel: 408-733-1055

2. Prepare 100X Thiolite™ Green stock solution:

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

Note: The unused Thiolite™ Green solution should be divided into single use aliquots, stored at -20°C and kept from light.

3. Prepare GSH reaction mixture:

Add 50 µL of 100X Thiolite™ Green stock solution (from Step 2) into 5 mL of assay buffer (Component B), and mix well.

4. Prepare serial dilutions of GSH standard (0 to 30 µM):

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

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

4.3 Add GSH standards and GSH-containing or other thiol-containing test samples into a solid black 96-well microplate as shown in Tables 1 and 2.
Note: Treat cells 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 serial dilutions of GSH standard from 0.01 µM to10 µM into wells from GS1 to GS7 in duplicate.*

5. Run GSH assay:

5.1 Add 50 µL of GSH reaction mixture (from Step 3.1) to each well of the GSH standard, blank control, and test samples (see Step 4.3) to make the total GSH assay volume of 100 µL/well.
Note: For a 384-well plate, add 25 µL of sample and 25 µL of GSH reaction mixture into each well.

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

5.3 Monitor the fluorescence increase at Ex/Em = 490/520 nm with a fluorescence plate reader.

Ordering: sales@aatbio.com; Tel: 800-990-8053 or 408-733-1055; Fax: 408-733-1304
Technical Support: support@aatbio.com; Tel: 408-733-1055

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. A GSH standard curve is shown in Figure 1.
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.

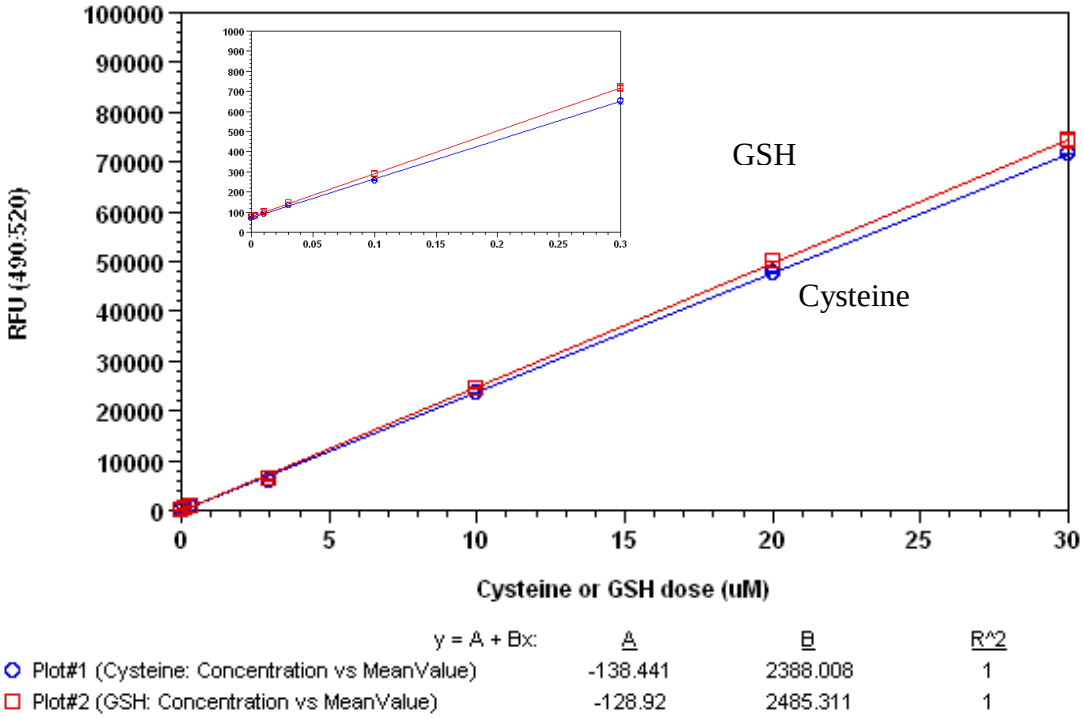

Figure 1 GSH and Cysteine dose responses were measured in a 96-well black plate with Amplite™ Fluorimetric Thiol Quantitation Assay Kit using a NOVOstar microplate reader (BMG Labtech). As low as 10 nM (1 pmol/well) of GSH or Cysteine can be detected with 10 minutes incubation time (n=3). The insert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

References

1. Mudd SH, Levy HL, Skovby F. Disorder of transsulfuration. In:Scriver CR, Beaudet AL, Sly WS, Valle D, eds. The metabolic andmolecular bases of inherited disease, 7th ed. New York: McGraw-Hill, 1995:1229–1327.
2. Meister A. Selective modification of glutathione metabolism. Science 1983;220:472–7.
3. Gahl WA, Bashan N, Tietze F, Bernardini I, Schulman JD. Lysosomal cystine transport is defective in cystinosis. Science 1982; 217:1263–5.
4. Segal S, Thier SO. Cystinuria. In: Scriver CR, Beaudet AL, Sly WS, Valle D, eds. The metabolic and molecular bases of inherited disease, 7th ed. New York: McGraw-Hill, 1995:3581–3601.
5. Gahl WA, Ingelfinger J, Mohan P, Bernardini I, Hyman PE, Tangerman A. Intravenous cysteamine therapy for nephropathic cystinosis. Pediatr Res 1995; 38:579–84.
6. Hautmann R, Terhorst B, Stuhlsatz HW, Lutzeyer W. Mercaptopropionylglycine: a progress in cystine stone therapy. J Urol 1977; 117:628.