

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 Quantitation 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 properties 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 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 into single use aliquots and stored at -20°C.

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.

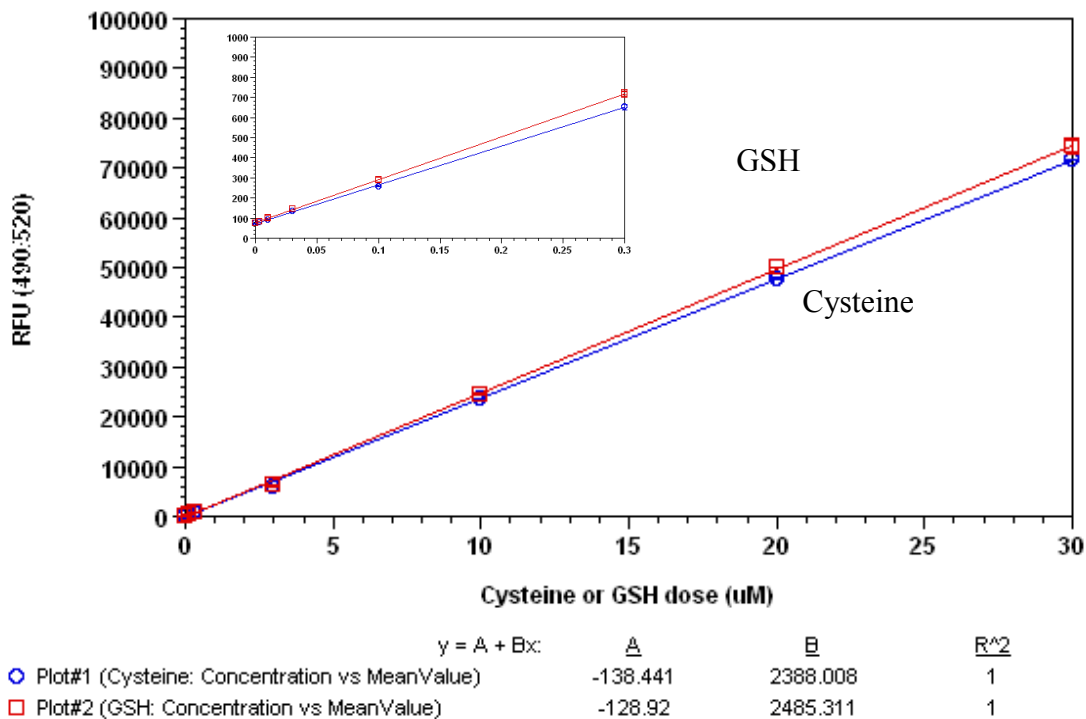


Figure 1 GSH and Cysteine dose responses were measured in a 96-well black plate with Amplite™ Fluorimetric Thiol Quantitation Assay Kit using a NOVostar microplate reader (BMG Labtech). As low as 10 nM (1 pmol/well) of GSH or Cysteine can be detected with 10 minutes incubation time (n=3). The insert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

References

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