

Amplite™ Fluorimetric Glutathione Assay Kit

Green Fluorescence

Ordering Information	Storage Conditions	Instrument Platform
Product Number: 10055 (200 assays)	Keep at -20 °C Avoid exposure to moisture and light	Fluorescence microplate readers

Introduction

Glutathione (GSH) is a tripeptide that contains L-cysteine, L-glutamic acid, and glycine. It is the smallest intracellular protein thiol molecule in cells, which regulates cell activity and prevents damage caused by reactive oxygen species such as free radicals and peroxides. The monitoring of reduced and oxidized GSH in biological samples is essential for evaluating the redox and detoxification status of the cells and tissues against oxidative and free radicals mediated cell injury. The detection and measurement of glutathione 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 glutathione content in biological systems, but all the commercial kits either lack sensitivity or have tedious protocols.

Our Amplite™ Fluorimetric Glutathione Assay Kit provides an ultrasensitive fluorimetric assay to quantitate GSH in sample. The proprietary non-fluorescent glutathione sensor used in the kit becomes strongly green fluorescent upon reacting with a GSH compound, which has the spectral properties almost identical to those of fluorescein and can be easily read by a fluorescence microplate reader at Ex/Em = 490/520 nm. The kit can detect as little as 1 picomole of GSH in a 100 µL assay volume (10 nM). In addition, both absorption and emission spectra of the glutathione adduct are pH-independent, making this assay kit highly robust. 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.

Kit Key Features

Broad Application:	Can be used for quantifying glutathione in a variety of biological systems (e.g., plasma, urine and cell extracts)
Sensitive:	Detect as low as 1 picomole of glutathione.
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)

5. Run GSH assay:

5.1 Add 50 μ L of GSH reaction mixture (from Step 3) into each well of GSH standard, blank control, and test samples (see Step 4.3) to make the total GSH assay volume of 100 μ L/well.

Note: For a 384-well plate, add 25 μ L of sample and 25 μ L 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 using a fluorescence plate reader.

Data Analysis

The fluorescence in blank wells (with the assay buffer only) is used as a control, and is subtracted from the values for the wells with the GSH reaction. 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.*

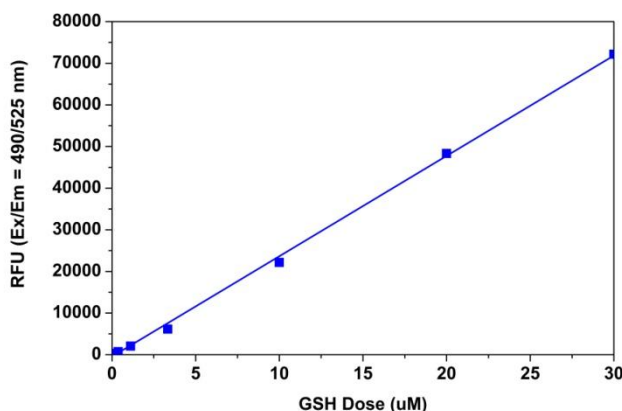


Figure 1 GSH dose responses were measured in a black 96-well plate with Amplite™ Fluorimetric Glutathione Assay Kit using a NOVostar microplate reader (BMG Labtech). As low as 10 nM (1 pmol/well) of GSH was detected with 10 minutes incubation (n=3).

References

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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.
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6. Hautmann R, Terhorst B, Stuhlsatz HW, Lutzeyer W. Mercaptopropionylglycine: a progress in cystine stone therapy. J Urol 1977; 117:628.

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