Elite™ Glutathione (GSH) Assay Kit (Green Fluorescence)

CATALOG NUMBER: CA-G055, 200 assays

Description

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.

The Elite™ 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 Components

- **Component A**: Thiolite™ Green — 1 vial
- **Component B**: Assay Buffer — 1 bottle (25 ml)
- **Component C**: GSH Standard — 1 vial (62 µg)
- **Component E**: DMSO — 1 vial (200 µl)
- **Component G**: Cell/Tissue Lysis Buffer — 1 bottle (10 ml)

Storage

Keep in freezer (-20 °C) and avoid exposure to light.

Shelf Life

All reagents are stable for at least 6 months after receipt when stored properly at the recommended conditions.

Materials required (but not supplied)

- 96 microplates: Tissue culture microplate with black wall and clear bottom is recommended.
- Fluorescence microplate reader.

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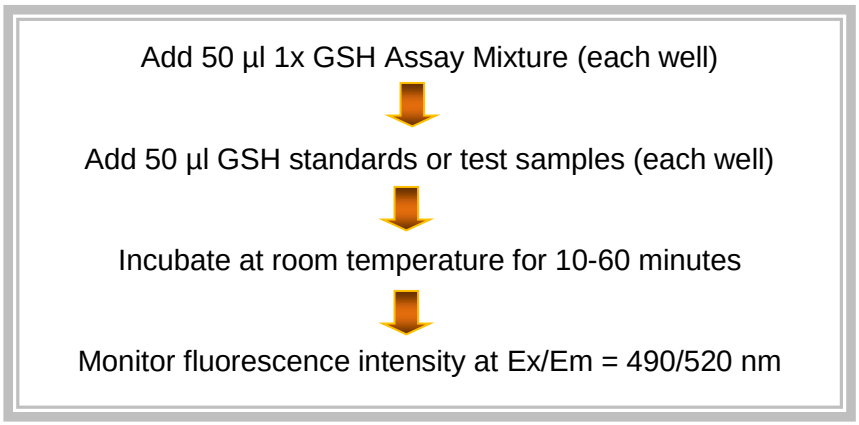
Assay Protocol (for 96-Well Plate)

Summary of the Assay



1. **Preparation of GSH standard stock solution:**

   Add 200 µl of Assay Buffer (**Component B**) into the vial of GSH Standard (**Component C**) to make 1 mM (1 nmol/µl) GSH standard stock solution.

   Note: The unused 1 mM GSH standard stock solution should be divided into single use aliquots and stored at -20 °C.

2. **Preparation of 100x Thiolite™ Green stock solution:**

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

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

3. **Preparation of 1x GSH assay mixture:**

   Add 50 µl of 100X Thiolite™ Green stock solution (from Step 2) into 5 ml of Assay Buffer (**Component B**), and mix them well.

4. **Prepare serially diluted GSH standards (0 to 30 µM):**

   4.1. Add 30 µl of GSH standard stock solution (from Step 1) into 970 µl of Assay Buffer (**Component B**) to generate 30 µM (30 pmol/µl) GSH standard solution.

   Note: Diluted 30 µM 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 serially diluted GSH standards.

   4.3. Add GSH standards and GSH-containing or other GSH-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 (***see Appendix A***).

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.

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Table 2. Reagent composition for each well

GSH Standards	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 to 10 µM into wells from GS1 to GS7 in duplicate.

5. **Run the GSH assay:**

5.1. Add 50 µl 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 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 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.

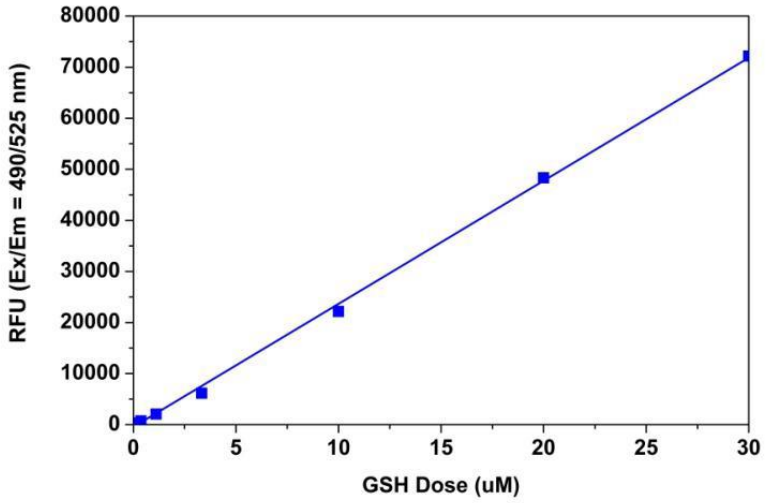

Figure 1. GSH dose responses were measured in a black 96-well plate with Elite™ Glutathione Assay Kit using a NOVOstar microplate reader (BMG Labtech). As low as 10 nM (1 pmol/well) of GSH was detected with 10-minute incubation (n=3).

References

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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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Appendix A

Sample cell lysis preparation for the assay kits

This protocol serves as a general guide for preparing samples for the assays. For the best results, users need to choose their own method for cell lysis preparation. ***A method of freeze-thaw (or other lysis methods using physical disruption in PBS) is strongly recommended for the preparation of cell lysate, especially when the background noise becomes an issue for this assay.***

1. Lysis of plant cells

   Homogenize the leaves with the Cell/Tissue Lysis Buffer (**Component G**) at 200 mg/ml, and centrifuge at 2500 rpm for 5-10 minutes. Use the supernatant for the assay.

2. Lysis of bacterial cells

   Collect bacterial cells by centrifugation (i.e. 10,000 x g, 0°C, 15 min). Add Cell/Tissue Lysis Buffer (**Component G**) to the pellet (1 mL per 100 to 10,000,000 cells), and leave at room temperature for 15 minutes. Centrifuge at 2500 rpm for 5 minutes, and use the supernatant for the assay.

3. Lysis of mammalian cells

   Remove the medium from the culture plate (wells). Use about 100 µL Cell/Tissue Lysis Buffer (**Component G**) per 1-5 million cells (or add lysis buffer 100 µL lysis buffer per well in a 96-well cell culture plate), and leave at room temperature for 15 minutes. For the assay, use the lysate directly or centrifuge at 1500 rpm for 5min then use the supernatant.

4. Lysis of tissues

   Weigh ~ 20 mg tissue, and then wash with cold PBS buffer. Homogenize the tissue with 400 µl of Cell/Tissue Lysis Buffer (**Component G**) in a micro-centrifuge tube, and centrifuge at 2500 rpm for 5-10 minutes. Use the supernatant for the assay.

   Note: use Cat# CA-N015g for ordering the Cell/Tissue Lysis Buffer (Component G) separately.

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