SensoLyte® Total GSH Assay Kit
Colorimetric

Revision Number: 1.1	*Last updated: October 2014*
Catalog #	**AS-72153**
Kit Size	500 Assays (96-well plate)

- ***Optimized Performance:*** Optimal conditions for the quantitation of total GSH.
- ***Enhanced Value:*** Ample reagents to perform 500 assays in a 96-well format.
- ***High Speed:*** Entire process can be completed in one hour.
- ***Assured Reliability:*** Detailed protocol and references are provided.

Kit Components, Storage and Handling

Component	Description	Quantity
Component A	Thiol Detection Reagent	1 mL
Component B	Reduced Glutathione Standard	10 mM, 200 µL
Component C	GSH Reductase	150 µL
Component D	NADPH	60 µL
Component E	Assay Buffer	100 mL
Component F	5-Sulfosalicylic Acid (SSA)	5 g

Other Materials Required (but not provided)

- 96-well microplate: Clear microplates provide better signal for absorbance reading.
- Microplate reader: Capable of detecting absorbance at 405 nm or 415 nm.

Storage and Handling

- Store Components A, B, and D at -20°C.
- Protect Component A from light and moisture.
- Store Components C and F at 4°C.
- Component E can be stored at room temperature for convenience.

Introduction

Reduced Glutathione (GSH) is the main thiol (sulfhydryl) compound in animal, plant and protozoa. GSH plays a critical role in cellular defense against oxidative stress and functions as a cofactor for a variety of enzymes such as glutathione transferase and glutathione peroxidase. GSH is also involved in the maintenance of redox status, amino acid transport and mitichondrial respiration.[1, 2] Total glutathione includes reduced form and oxidized form (GSSG). Upon oxidation, GSH is converted to GSSG, with the ratio of GSH/GSSG decreasing due to GSSG accumulation. The concentrations of GSH and GSSG and their molar ratio are indicators of the cell's functionality and oxidative stress level.[3, 4]

The SensoLyte® Glutathione Assay Kit provides a simple and convenient colorimetric assay for measurement of total GSH (GSH+GSSG). Glutathione reductase converts oxidized glutathione to reduced glutathione. Upon reaction with reduced GSH, Ellman's Reagent [5, 5'-dithiobis (2-nitrobenzoic acid)] supplied in the kit, releases a colored product, monitored at 415 nm using a microplate reader. The intensity of the color produced is proportional to GSH concentration. The kit also provides sulfosalicylic acid for deproteination of biological samples.

Protocol

Note: Avoid reducing agents (e.g. dithiothreitol, DTT; β-mercaptoethanol) and thiol alkylating agents (e.g. N-ethylmaleimide) in test samples. Phosphate buffered saline (PBS) is not provided.

1. Prepare biological samples

1.1 Cell lysate

- Collect cells by centrifugation at 2500 rpm for 5 min.
- Resuspend cell pellets in PBS.
- Perform 3 freeze-thaw cycles to lyse cells.
- Centrifuge cell lysate for 10 min at 12,000 rpm at 4°C.
- Collect the supernatant, aliquot, and keep on ice.
- Measure protein concentration of cell lysate.

Note: Measure protein concentration based on absorbance at 280nm or use Dye Reagent Concentrate (Bio-Rad Protein Assay, Cat#500-0006)

1.2 Deproteination

- Prepare 5% SSA solution. Dissolve the SSA powder (Component F) in 100 mL distilled water. Ensure the powder is thoroughly dissolved. Keep at 4°C.
- Using this solution (5% SSA solution), add 4 times the volume of the cell lysate, vortex.
- Centrifuge the mixture of cell lysate and 5% SSA at 12,000 rpm for 5 min at 4°C.
- Collect supernatant without disturbing the precipitate and use this as the sample to detect GSH.
- Aliquot and keep deproteinated supernatant in -80°C, if the assay is not performed on the same day.

2. Prepare working solutions.

Note: Warm all kit components until thawed to room temperature before starting the experiments.

2.1 GSH reaction mixture: Prepare GSH reaction mixture according to Table 1. For each experiment, prepare fresh reaction mixture.

Note: To measure only the reduced GSH, do not add the GSH Reductase and NADPH to the detection solution.

Table 1. GSH reaction mixture for one 96-well plate (100 assays)

Components	Volume
Thiol detection reagent (Component A)	180 µL
GSH Reductase (Component C)	30 µL
NADPH (Component D)	11 µL
Assay buffer (Component E)	8.779 mL
Total volume	9 mL

2.2 Prepare GSH standard solution: Dilute the GSH standard stock solution (Component B) 1:10 to 1000 µM in 5% SSA. Do 2-fold serial dilutions to get concentrations of 500, 250, 125, 63, 32 and 16 µM. Include a blank control of 5% SSA alone.

3. Set up the reaction.

3.1 Add 1-10 µL of test sample into microplate wells.

Note 1: Use assay buffer (Component E) to dilute test samples.

Note 2: If the samples are diluted in buffers containing substances that may affect assay performance, test the same amount of that buffer with glutathione standards.

3.2 Set up the GSH standard: Add 10 µL of serially diluted GSH standard solution.

3.3 Bring the total volume of all controls and samples to 10 µL.

4. Run the reaction.

4.1 Add 90 µL of GSH reaction mixture into each well. Mix the reagents completely by shaking the plate gently for 30 sec.

4.2 Measure signal:

- For kinetic reading: Immediately start measuring absorbance at 405 or 415 nm continuously and record data every 5 min. for 30 min.
- For end-point reading: Incubate the reaction for 30 min. Keep plate from direct light. Mix the reagents and measure absorbance at 405 or 415 nm.

5. Data Analysis.

5.1 The absorbance reading from the blank control well is used as the background absorbance. This background reading should be subtracted from the readings of the other wells containing thiol detection reagent.

5.2 Plot GSH standard curve as absorbance versus glutathione concentration and determine the linear regression.

Note: The final concentrations of GSH standard are 100, 50, 25, 12, 6, 3, 1.5, and 0 µM.

5.3 Use the equation of GSH standard curve for calculation of GSH level in test samples:

$$[\text{Total GSH}] = \frac{((\text{Abs}_{\text{sample}} - \text{Abs}_{\text{blank}}) - \text{Y-intercept})}{\text{Slope}_{\text{standard curve}}} \text{ X Dilution Factor}$$

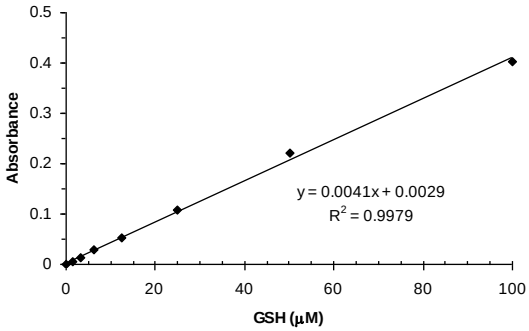

Figure 1. GSH reference standard. Serial dilutions of GSH were incubated with GSH reaction mixture 30 minutes and absorbance was measured at 405 nm (Ultra Microplate Reader EL808, Bio-Tek Instruments, Inc).

5.4 If using kinetic reading prepare GSH standard curve plotting velocities values (V) for standards as a function of GSH concentration. Velocity value from the blank control well is used as the background and should be subtracted from velocities of the other samples. Velocities are expressed as the change of absorbance per minute, ΔAbs/min.

Use the following equation of GSH standard curve for calculation of GSH level in test samples:

$$[\text{Total GSH}] = \frac{((\text{V}_{\text{sample}} - \text{V}_{\text{blank}}) - \text{Y-intercept})}{\text{Slope}_{\text{standard curve}}} \text{ X Dilution Factor}$$

Note: For direct, quantitative comparison of data obtained in independent experiments, prepare a separate calibration curve for each test series.

References

1. Dickinson, DA. et al. *Ann. N. Y. Acad. Sci.* **937**, 488 (2002).
2. Townsend, DM. et al. *Biomed. Oharmacother.* **57**, 145 (2003).
3. Ballatori, N. et al. *Biol. Chem.* **390**, 191 (2009).
4. Rahman, I et al. *Antioxid. Redox Signal.* **7**, 42 (2005).