SensoLyte® 520 Total GSH Kit
Fluorimetric

Revision Number: 1.1	*Last updated: October 2014*
Catalog #	**AS-72154**
Kit Size	100 Assays (96-well plate)

- ***Optimized Performance:*** Optimal conditions for the quantitation of total GSH.
- ***Enhanced Value:*** Ample reagents to perform 100 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	25 µL
Component B	Reduced Glutathione Standard Stock Solution	10 mM, 100 µL
Component C	GSH Reductase	30 µL
Component D	NADPH	15 µL
Component E	Assay Buffer	20 mL
Component F	5-Sulfosalicylic Acid (SSA)	1 g

Other Materials Required (but not provided)

- 96-well microplate: Black, flat-bottom, non-binding 96-well plate.
- Fluorescence microplate reader: Capable of detecting emission at 520 nm with excitation at 490 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 crucial 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 mitochondrial respiration.[1, 2] Total glutathione includes the 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® 520 Total GSH Kit provides a convenient and highly sensitive fluorescent assay for the measurement of total GSH (GSH+GSSG). This kit contains a novel fluorogenic reagent, which upon reaction with reduced GSH releases fluorescence. Fluorescence is monitored at excitation/emission = 490/520 nm. GSSG can be converted to the GSH by glutathione reductase. The assay has a linear range of 0.2-100 μM of GSH. 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 280 nm 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 20 mL distilled water. Ensure that 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 cell lysate with 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 at -80°C, if the assay is not performed on the same day.

2. Prepare working solutions.

Note: Thaw all kit components 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 reduced GSH only, do not add 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)	25 µL
GSH Reductase (Component C)	30 µL
NADPH (Component D)	11 µL
Assay buffer (Component E)	8.934 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 (from Step 2.2) to the wells.

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 fluorescence signal:

- For kinetic reading: Immediately start measuring fluorescence intensity at Ex/Em=490/520 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 fluorescence intensity at Ex/Em=490/520 nm.

Note: Fluorescence signal is stable at room temperature for at least 2 hours.

5. Data analysis.

5.1 The fluorescence reading from the blank control well is used as the background fluorescence. This background reading should be subtracted from the readings of the other wells containing GSH detection reagent. All fluorescence readings are expressed in relative fluorescence units (RFU).

5.2 Plot GSH standard curve as RFU 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:

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

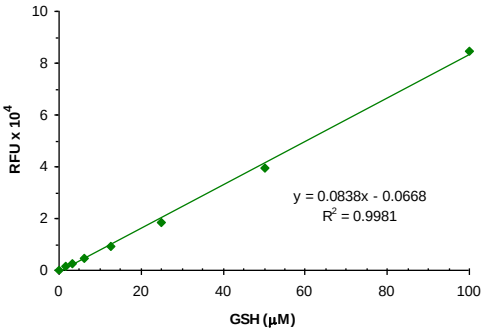

Figure 1. Glutathione standard curve. Serial dilutions of GSH were incubated with GSH reaction mixture. Fluorescence was measured at 30 min. at Ex/Em=490/520 nm (FlexStation 384II).

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 in RFU/sec.

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

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

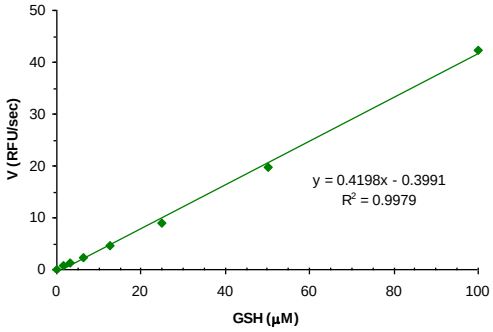

Figure 2. Glutathione standard curve for kinetic reading. Plot of velocity (V) of reaction versus GSH concentration. Fluorescence signal was monitored at Ex/Em=490/520 nm (FlexStation 384II).

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).