SensoLyte® Thiol Quantitation Kit
Colorimetric

Revision Number: 1.1	*Last updated: October 2014*
Catalog #	**AS-72136**
Kit Size	500 Assays (96-well plate)

- ***Optimized Performance:*** Optimal conditions for the quantitation of thiol.
- ***Enhanced Value:*** Ample reagents to perform 500 assays in a 96-well format.
- ***High Speed:*** Entire process can be completed in 30 minutes.
- ***Assured Reliability:*** Detailed protocol is provided.

Kit Components, Storage and Handling

Component	Description	Quantity
Component A	Thiol Detection Reagent	1 mL
Component B	Reduced Glutathione (GSH) Standard	10 mM, 100 µL
Component C	Assay Buffer	100 mL

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 all kit components at -20°C
- Protect Component A from light and moisture
- Component C can be stored at room temperature for convenience

Introduction

Thiol compounds, such as glutathione (GSH), cysteine, and homocysteine, are a natural reservoir of the reductive capacity of a cell. They function as components of the intracellular and extracellular redox buffer and play important roles in a variety of biological processes, such as enzyme catalysis, redox-signaling protein folding, and free radical scavenging.[1-3]

The SensoLyte® Thiol Quantification Kit provides a simple and convenient colorimetric assay for measuring thiol in biological samples. The sulfhydryl group of thiol reacts with Ellman's Reagent [5, 5'-dithiobis (2-nitrobenzoic acid)]. The final product of this reaction, 2-nitro-5-thiobenzoic acid (TNB) has yellow color, which can be detected at 415 nm using a spectrophotometric microplate reader. The assays are performed in a convenient 96-well microplate format.

Protocol

Note: Avoid reducing agents (e.g. DTT, β-mercaptoethanol) in test samples.

1. Prepare working solutions.

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

1.1 Thiol detection reagent solution: Dilute Thiol detection reagent (Component A) in assay buffer (Component C) according to Table 1. For each experiment prepare fresh detection solution.

Table 1. Thiol detection reagent solution for one 96-well plate (100 assays).

Components	Volume
Thiol detection reagent (Component A)	200 µL
Assay buffer (Component C)	9.8 mL
Total volume	10 mL

1.2 Prepare dilutions of GSH standard: Dilute the GSH (Component B) 10-fold to 1 mM in assay buffer (Component C). Do 2-fold serial dilutions to get concentrations of 500, 250, 125, 62.5, 31, 15.5 µM. Include a blank control.

2. Set up the thiol reaction.

2.1 Add 1-10 µL of test sample into microplate wells

Note 1: Use assay buffer (Component C) 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.

2.2 Set up GSH standards: Add 10 µL serially diluted GSH reference solutions (from step 1.2) to the wells.

2.3 Bring the total volume of all samples to 10 µL.

3. Run the reaction.

3.1 Add 90 µL of thiol detection reagent solution into each well. Mix the reagents completely by shaking the plate gently for 30 sec.

3.2 Measure the signal: Incubate the reaction for 10 to 30 min at room temperature. Keep plate from direct light. Read absorbance at 405 nm or 415 nm using a microplate reader.

4. Data Analysis.

4.1 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.

4.2 Plot GSH standard curve as absorbance versus glutathione concentration and determine the linear regression (Fig. 1).

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

4.3 Use glutathione standard curve for calculation of thiol level in test samples.

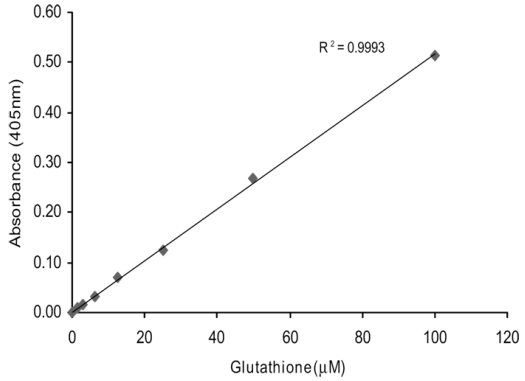

Figure 1. GSH reference standard. Serial dilutions of GSH were mixed with thiol detection solution and absorbance was measured at 405 nm (Ultra Microplate Reader EL808, Bio-Tek Instruments, Inc). Detection limit can reach as low as 1.5 µM of GSH.

References

1. Dickinson, DA. et al. *Biochem. Pharmacol.* **64**, 1019 (2002).
2. Winterbourn, CC. et al. *Free Radical Biol. Med.* **45**, 549 (2008).
3. Franco, R. et al. *Arch. Physiol. Biochem.* **113**, 234 (2007).