



SensoLyte[®] 520 Thiol Quantitation Kit

Fluorimetric

Revision Number: 1.1	<i>Last updated: October 2014</i>
Catalog #	AS-72138
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 half an hour.
- **Assured Reliability:** Detailed protocol is provided.

Kit Components, Storage and Handling

Component	Description	Quantity
Component A	Thiol Detection Reagent	500 μ L
Component B	Reduced Glutathione Standard Stock Solution	10 mM, 20 μ L
Component C	Assay Buffer	50 mL

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 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[®] 520 Thiol Quantitation Kit provides a convenient, highly sensitive fluorescent assay for measurement of total thiol level in various samples. This kit contains a novel fluorogenic reagent which upon reaction with thiol releases fluorescence. Fluorescence signal can be detected at excitation/emission=490 nm/520 nm. The assay has a linear range of 0.04-160 μ M thiol. Common contaminants, e.g. DMSO, and Triton X-100, are well tolerated in the assay.

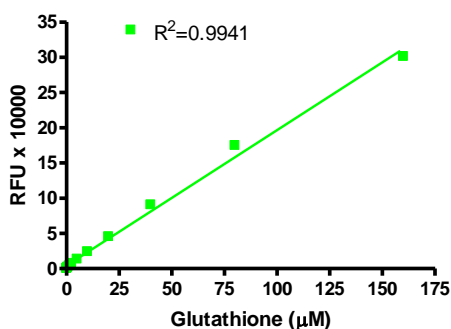


Figure 1. Detection of GSH with SensoLyte[®] 520 Quantitation Kit. Fluorescence signal was detected at Ex/Em=490/520 nm (FlexStation 384II).

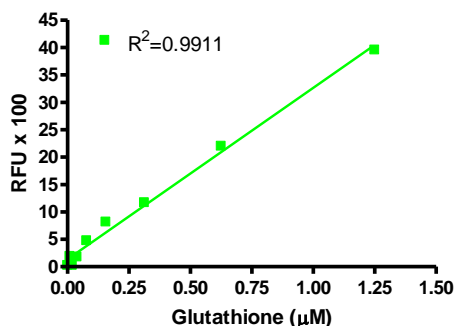


Figure 2. Expanded lower concentration range using the SensoLyte[®] 520 Thiol Quantitation Kit. Fluorescence signal was detected at Ex/Em=490/520 nm (FlexStation 384II).

Protocol

Note: Avoid reducing agents (e.g. dithiothreitol, 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)	100 μ L
Assay buffer (Component C)	9.9 mL
Total volume	10 mL

1.2 Prepare GSH standard solution: Dilute the GSH stock solution 1:50 to 200 μ M in assay buffer (Component C). Do 2-fold serial dilutions to get concentrations of 100, 50, 25, 12.5, 6.3, and 3.2 μ M. Include a blank control.

2. Set up the thiol reaction.

2.1 Add 1-10 μ L 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 the GSH standard: Add 10 μ L serially diluted GSH standard solution (from Step 1.2) to the wells.

2.3 Bring the total volume of all controls and samples to 10 μ L.

3. Run the reaction.

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

3.2 Measure fluorescence signal: Incubate the reaction for 5 to 30 min. Keep plate from direct light. Measure fluorescence intensity at Ex/Em=490/520 nm. Fluorescence signal is stable at room temperature for at least 2 hours.

4. Data analysis.

4.1 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 thiol detection reagent. All fluorescence readings are expressed in relative fluorescence units (RFU).

4.2 Plot GSH standard curve as RFU versus glutathione concentration and determine the linear regression.

Note: The final concentrations of GSH standard are 20, 10, 5, 2.5, 1.2, 0.6, 0.3, and 0 μ M.

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

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).