SensoLyte® ABD-F Thiol Quantitation Kit
Fluorimetric

Revision Number: 1.1	*Last updated: October 2014*
Catalog #	**AS-72137**
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	500 µL
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: Black, flat-bottom, non-binding 96-well plate.
- Fluorescence microplate reader: Capable of detecting emission at 513 nm with excitation at 389 nm

Storage and Handling

- Store all kit components at -20°C
- Protect Components A and B 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® ABD-F Thiol Quantification Kit provides a convenient, sensitive fluorescent assay for measuring thiol in biological samples. This kit contains ABD-F (4-Fluoro-7-aminosulfonylbenzofurazan), a fluorogenic reagent, which readily reacts with thiol compounds to generate highly fluorescent products. The fluorescent signal can be detected at excitation/ emission=389nm/513nm.

Protocol

Note: Avoid reducing agents (e.g. DTT, β-mercaptoethanol) in test samples. Warm all reagents to room temperature before use.

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) 1:100 in assay buffer (Component C) according to Table 1. For each experiment prepare fresh reagent solution.

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

Components	Volume
Thiol detection reagent (Component A)	100 µL
Assay buffer (Component C)	9.9 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 controls to 10 µL.

3. Run the reaction.

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

3.2 Measure the fluorescence signal: Incubate the reaction for 30 min at room temperature. Keep plate from direct light. Measure fluorescence intensity at Ex/Em=389/513nm. 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 substrate. 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 (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.

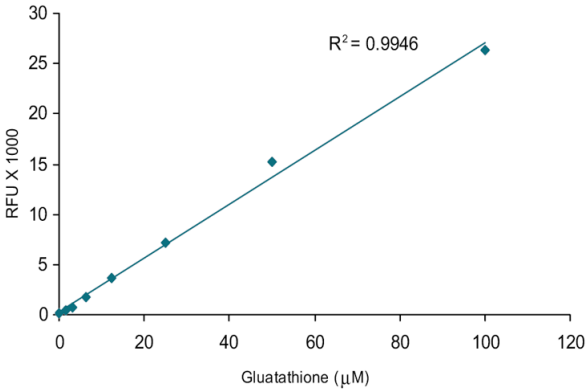

Figure 1. GSH reference standard. Serial dilutions of GSH were mixed with substrate solution. Fluorescence signal was detected at Ex/Em=389nm/513nm (FlexStation 384II, Molecular Devices). The detection limit can reach as low as 0.25 μM of GSH.

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).