SensoLyte® Glutathione Cellular Assay Kit *Fluorimetric*

Revision Number: 1.1	*Last updated: October 2014*
Catalog #	**AS-72158**
Kit Size	100 Assays (96-well plate)

- ***Optimized Performance:*** Optimal conditions for detection of 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	GSH detection reagent	1 vial
Component B	Reduced Glutathione standard	10 mM, 100 µL
Component C	Assay Buffer	50 mL
Component D	GST enzyme	50U/mL, 200 µL
Component E	DMSO	100 µL

Other Materials Required (but not provided)

- Microplate: Black, flat-bottom, 96-well plate with non-binding surface.
- Fluorescence microplate reader: Capable of detecting emission at 480 nm with excitation at 390 nm.

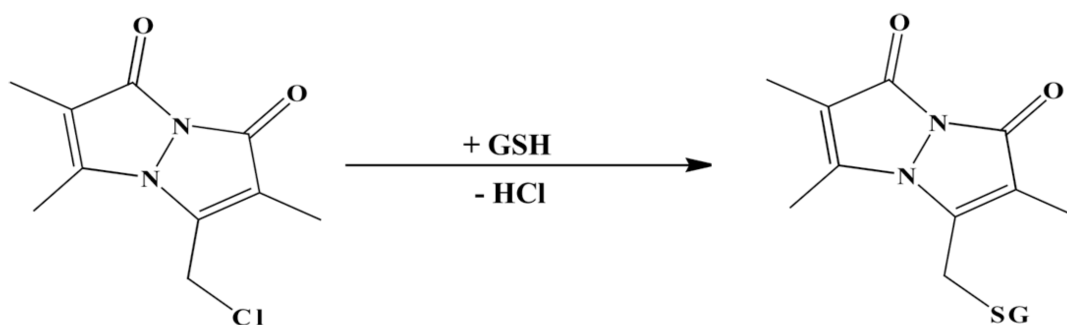
Storage and Handling

- Store Components A and B at -20°C.
- Store Component D at -80°C. Aliquot as needed to avoid multiple freeze-thaw cycles.
- Components C and E can be stored at room temperature for convenience.
- Protect Components A from light and moisture.

Introduction

Glutathione (GSH), a low molecular weight thiol, plays an important role in antioxidant defenses, heavy metal tolerance and xenobiotic detoxification.[1] As a prototype antioxidant, it has been shown to be involved in cell protection from the noxious effect of excess oxidant stress, both directly and as a cofactor of glutathione peroxidases.[2] Variation observed in glutathione levels is a good indicator of oxidative stress in tissues.[3]

The Sensolyte® Glutathione Cellular Assay Kit provides a convenient and sensitive fluorescent assay for determination of the glutathione levels in cell or tissue extracts. The kit contains a non-fluorescent substrate, which selectively reacts with GSH in reaction catalyzed by glutathione S-transferase (GST) to form a fluorescent glutathione adduct. The resulting blue fluorescence can be monitored at excitation /emission= 390 nm/480 nm. The kit provides reagents sufficient for 100 assays (96-well plate).



Scheme 1. Enzymatic conversion of non-fluorescent monochlorobimane to a fluorescent glutathione adduct by glutathione *S*-transferase.[4]

Protocol

1. Prepare biological samples.

1.1 Prepare cell extract samples:

- Induce apoptosis in cell culture using a method of your choice. Prepare control cells without apoptosis induction.
- Culture cells in a 37°C incubator for the desired exposure time.
- Collect cells by centrifugation at 1500 rpm for 5-10 min.
- Resuspend cell pellets in PBS.
- Perform 3 freeze-thaw cycles to lyse cells.
- Centrifuge cell lysate for 15 min at 10,000xg at 4°C.
- Collect the supernatant and store at -70°C until use.

2. Prepare working solutions.

Note: Bring the kit components to room temperature

2.1 GSH detection solution: Reconstitute the vial of Component A with 60 µL of DMSO (Component E). Dilute reconstituted Component A 1: 100 in assay buffer (Component C) according to Table 1. For each experiment, prepare fresh GSH detection solution.

Note: The stock solution of GSH detection reagent is good for 1-2 weeks if stored at -20°C.

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

Components	Volume
GSH detection reagent (Component A)	50 µL
Assay buffer (Component C)	4.95 mL
Total volume	5 mL

2.2 GST diluent: Dilute GST enzyme (Component D) 1:5 in assay buffer (Component C). Adjust the amount of enzyme to be diluted as required.

2.3 GSH reference standard: Dilute 10 mM GSH (Component B) 1:10 to 1 mM with assay buffer. Do 1:2 serial dilutions to get concentrations of 500, 250, 125, 62.5, 31.25 and 15.6 µM. Include a blank control.

3. Set up enzymatic reaction.

3.1 Add 1-40 µL of GSH containing test sample per well.

3.2 Add 10 µL of serially diluted GSH standard solution (from Step 2.3) to the wells.

3.3 Add 10 µL of GST diluent per well.

3.4 Bring the total volume of all controls to 50 µL in each well with assay buffer.

4. Run the enzymatic reaction.

4.1 Add 50 µL of GSH detection solution from Step 2.1 into each well. Mix the reagents completely by shaking the plate gently for 30 sec.

4.2 Measure fluorescence signal: Incubate the reaction for 30-60 min. Keep plate from direct light. Measure fluorescence intensity at Ex/Em=390 nm/480 nm.

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 substrate. All fluorescence readings are expressed in relative fluorescence units (RFU).

5.2 For data analysis, plot data as RFU versus activity of GSH standard and determine the linear regression (Fig. 1). Use GSH standard curve for calculation of GSH activity in test samples.

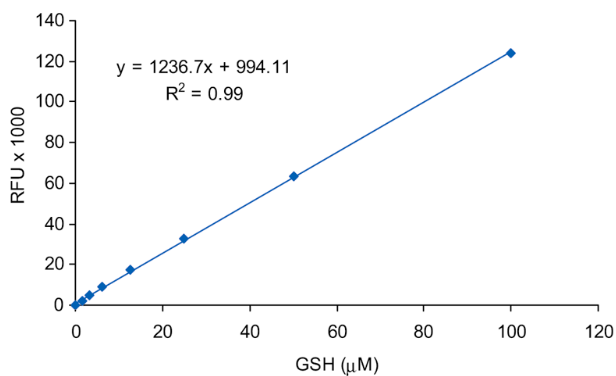


Fig.1. GSH reference standard. GSH was serially diluted in assay buffer containing detection reagent and GST, and the fluorescence recorded at Ex/Em-390/480 nm (Flexstation 384II, Molecular Devices).

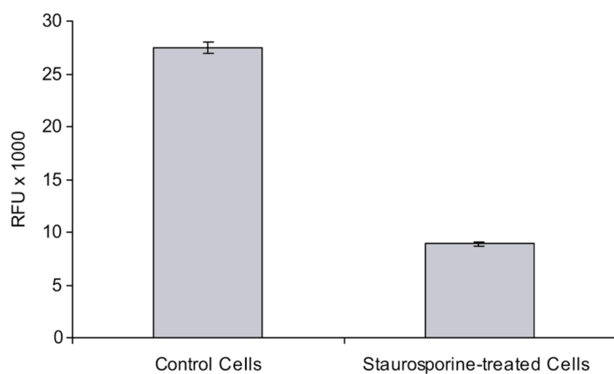


Fig.2. Measurement of glutathione level after apoptosis induction in HeLa cells with SensoLyte® Glutathione Cellular Assay Kit.

References :

1. Meter A. J. et al. *Plant J.* **1**, 27 (2001).
2. Pompella A. et al. *Biochem. Pharmacol*. **8**, 66 (2003).
3. Salehi P. et al. *Am. J. Transplant.* **4**, 5 (2004).
4. Nauen R. et al. *Anal. Biochem.* **2**, 303 (2002).