SensoLyte® GST Activity Assay Kit
Fluorimetric

Revision Number: 1.1	*Last updated: October 2014*
Catalog #	**AS-72157**
Kit Size	100 Assays (96-well plate)

- ***Optimized Performance:*** Optimal conditions for detection of GST activity.
- ***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	GST Substrate	1 vial
Component B	GST Standard	10 U/mL, 40 µL
Component C	Assay buffer	50 mL
Component D	Reduced Glutathione	20 mM, 300 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 D at -20°C.
- Store Component B 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 Component A from light and moisture.

Introduction

Glutathione S-transferases (GST) belong to a family of enzymes that is involved in detoxification and toxification mechanisms. They act via conjugation of reduced glutathione (GSH) with numerous substrates such as pharmaceuticals and environmental pollutants.[1-4] GST enzymes are believed to be crucial to the body's defense against cancer-causing chemicals and other toxins. These enzymes also carry out a wide range of functions in cells such as removal of reactive oxygen species (ROS) and regeneration of S-thiolated proteins, both of which are consequences of oxidative stress.

The SensoLyte® GST Activity Assay Kit provides a convenient and sensitive fluorescent assay for the detection of total GST activity in plasma, cell and tissue lysates. This kit contains a non-fluorescent substrate for GST, which upon conjugation with reduced glutathione generates bright fluorescence that can be detected at excitation/emission = 390nm/480 nm. The kit provides reagents sufficient for 100 assays (96-well plate).

Protocol

1. Prepare biological samples.

1.1 Cell lysates

- Wash cells with PBS.
- Add an appropriate amount of assay buffer (Component C) to cells or cell pellet. Collect the cell suspension in a microcentrifuge tube.
- Incubate the cell suspension for 10 minutes at 4°C.
- Centrifuge the cell suspension for 15 minutes at 10,000xg, 4°C. Collect the supernatant and store at -70°C until use.

1.2 Tissue samples

- Homogenize tissue samples in assay buffer (Component C).
- Incubate for 10 min. at 4°C.
- Centrifuge for 15 min. at 10,000xg at 4°C and collect the supernatant. Store at -70°C until use.

2. Prepare working solutions.

Note: Bring all kit components to room temperature before starting the experiments.

2.1 GST substrate solution: Reconstitute the vial (Component A) with 30 µL of DMSO (Component E). Dilute substrate solution 1:100 in assay buffer (Component C) according to Table 1. For each experiment, prepare fresh substrate solution.

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

Table 1. GST substrate solution for one 96-well plate (100 assays)

Components	Volume
GST substrate (Component A)	25 µL
Assay buffer (Component C)	2.475 mL
Total volume	2.5 mL

2.2 Reduced Glutathione: Dilute 20 mM GSH (Component D) 1:10 to 2 mM with assay buffer (Component C). Adjust the amount of GSH to be diluted as required.

2.3 GST Diluent: Dilute the enzyme (Component B) 1:10 in assay buffer (Component C) to get 1U/mL. Do 2-fold serial dilutions to get concentrations 500, 250, 125, 62, 31 and 15 mU/mL. Include a blank control.

3. Set up enzymatic reaction.

3.1 Add 50 µL of GST containing test sample per well.

3.2 Add 50 µL of serially diluted GST standard solution (from Step 2.3) to the wells.

3.3 Add 25 µL of GSH diluent per well.

3.4 Bring the total volume of all controls and samples to 75 µL in each well with assay buffer.

4. Run the enzymatic reaction.

4.1 Add 25 µL of GST substrate solution from Step 2.1 into each well. For better results, it is advisable to have the glutathione substrate solution equilibrated to the assay temperature. 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=390/480 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. 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 GST standard and determine the linear regression (Fig. 1). If using kinetic reading plot velocities (RFU/min/mg) versus activity of GST standard.

5.3 Use GST standard curve for calculation of GST activity in test samples

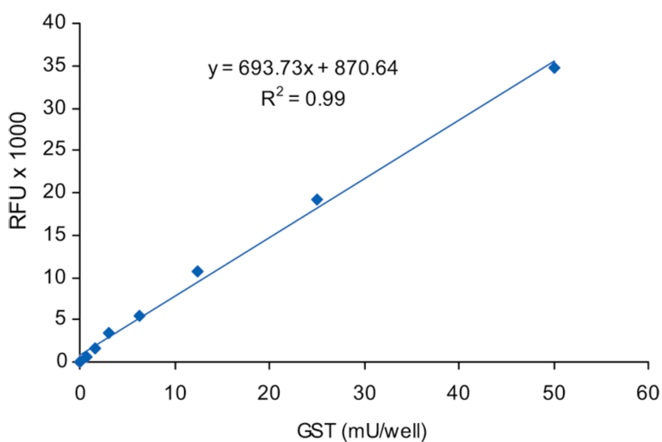

Figure 1. GST standard. Serially diluted GSTs were incubated with substrate and GSH, and the fluorescence recorded at Ex/Em=390 nm/480 nm. (Flex station 384II, Molecular Devices).

References

1. Sheenan D. et al. *Biochem J.* **360**, 1 (2001).
2. Rushmore T. et al. *J. Biol. Chem*. **268**, 11475 (1993).
3. Estonius M. et al. *Eur. J. Biochem.* **260**, 409 (1999).
4. Hayes D. J et al. *Annu. Rev. Pharmacol. Toxicol.* **45**, 51 (2005).