Rev. 1

Glutathione S-transferases Assay (GST)

Catalog #8548
100 Tests in 96-well plate

Product Description
Glutathione S-transferases (GST) comprise a family of enzymes that plays an important role in cellular detoxification of xenobiotics. GST catalyzes the conjugation of reduced glutathione to electrophilic xenobiotics and utilizes reduced glutathione to scavenge potentially toxic compounds. GST activity measurement has been used as biomarker for diagnoses of tumor and liver damage and oxidative stress. This colorimetric assay is based on the GST-catalyzed reaction between reduced glutathione and GST substrate, 1-chloro-2, 4-dinitrobenzene (CDNB). The GST activity is determined by measuring the rate of produced conjugation between reduced glutathione and CDNB, which is proportional to the increase in absorbance at 340nm over time (ΔOD_{340nm}/min).

Kit Components

Cat. No.	# of vials	Reagent	Quantity	Storage
8548a	1	Assay buffer	25mL	4°C
8548b	1	GST positive control	1 vial	-20°C
8548c	1	Substrate	0.2 mL	-20°C
8548d	1	Glutathione	2 vials	-20°C
8548e	1	Reconstitution Solution	1	4°C

Materials supplied by user:
Colorimetric microplate reader
96-well plate

Product Use
The GST Assay kit measures the total GST activity of different types of samples, such as tissues and cell lysate and plasma. This product is for research purposes only and not for use in animals, humans, or diagnostic procedures.

Quality Control
GST positive control is measured with the GST Assay kit after different time of reaction (Figure 1 and 2). The detection limitation is from 0.04 to 0.6 U/mL.

Shipping
Dry ice.

Reagents and Positive Control Preparation

1. Prior to use, ensure that all components are allowed to reach room temperature.
2. Centrifuge the GST positive control at 1,500x g for 1 minute.
3. Reconstitute GST positive control with 30 µl Reconstitution solution (Cat. #8548e).
4. Add 3 µl of reconstituted GST positive control into 37 µl assay buffer (Cat. #8548a). Prepare diluted GST positive control to a final volume of 10 µL/well on the 96-well flat bottom plate.
5. To prepare glutathione solution, add 275 µl of assay buffer (Cat. #8548a) into each vial (Cat. #8548d) immediately before use.
   Note: One 8548d vial is adequate for 50 assays. Any unused solution can be stored at –20°C for up to one week.

Procedure (96-well plate)

A. Preparation of test samples and blank

1. Cell or tissues can be homogenized in 4 volumes of the assay buffer (Cat. #8548a). Centrifuge the samples at 10,000 ×g for 10 minutes at 4ºC to remove insoluble material. The soluble fraction may be assayed directly.
2. Samples should be serial diluted to make sure the readings are within the standard curve range. Prepare test samples to a final volume of 10 µL/well on the 96-well flat bottom plate.
3. Prepare blank by adding 10 µL assay buffer (Cat. #8548a) into one well on the 96-well flat bottom plate.

B. Working reagent preparation and measurements

1. Prepare appropriate volume of GST assay working reagent based on the number of samples to be measured. For each well of reaction, prepare working reagent by mixing 83 µL assay buffer (Cat. #8548a), 2µL substrate (Cat. #8548c) and 5 µL glutathione solution. Vortex the solution shortly.
   Note: The solution would become slightly cloudy upon the addition of substrate to the solution.

2. Add 90 µL of working reagent mix into each well of the 96-well plate containing diluted GST positive control, samples and blank, mix well immediately and start recording OD_{340nm} over a 4-minute interval, collecting data every 30 seconds. Figure 1 shows data of diluted GST positive control.

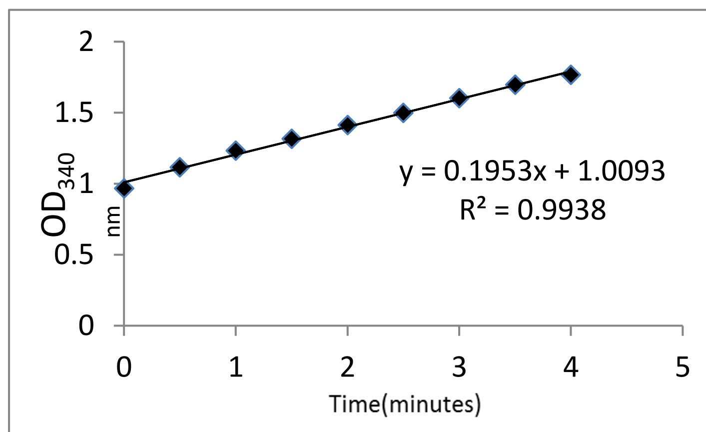

Figure 1 *Absorbance change of diluted GST positive control at 340nm.*

C. Calculations

1. Determine the change in absorbance ΔOD_{340nm}/min by plotting the absorbance value at ΔOD_{340nm} as a function of reaction time to obtain the slope of the linear portion of the curve. As shown in Figure 2.

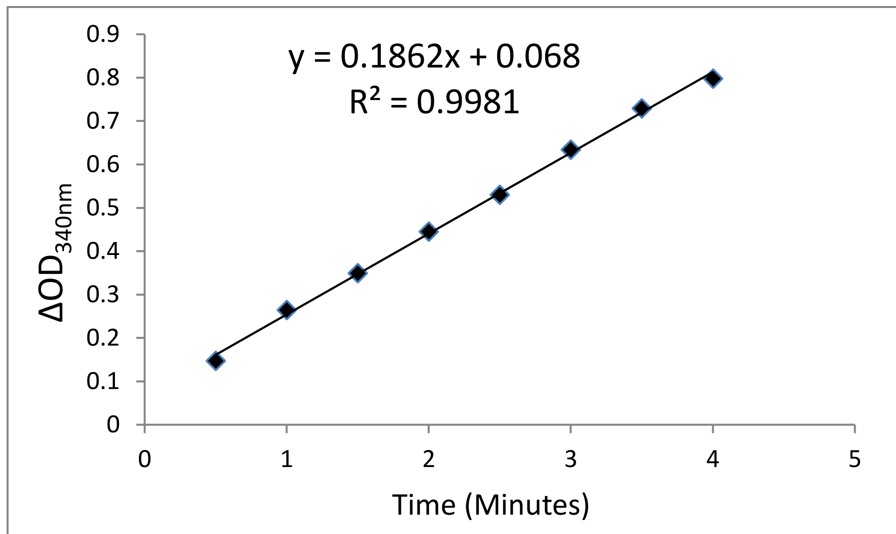

Figure 2 *The change in absorbance ΔOD340nm of diluted GST positive control during the time at 340nm.*

2. Calculate the GST activity using the following formula:

$$\text{GST positive control} = \frac{\Delta OD_{340nm}/\text{min} \times 100\ \mu l}{2.99mM^{-1} \times 10\ \mu l} \times \text{sample dilution}$$

Note: The actual extinction coefficient for CDNB at 340nm is $9.6mM^{-1}cm^{-1}$. This value has been adjusted for the path length of the solution in the 96-well plate.

Unit definition: One unit would conjugate 1.0 µmol CDNB to reduced glutathione per minute at 25 ºC

3. Use the formula to calculate GST positive control activity:

$$\text{GST positive control} = \frac{0.1862 \times 100\ \mu l}{2.99mM^{-1} \times 10\ \mu l} \times 40 = 24.909\ (U/ml)$$