KAMIYA BIOMEDICAL COMPANY

GST Fluorometric Activity Assay

For the quantitative determination of GST activity in plasma, cell and tissue homogenates

Cat. No. KT-205

For Research Use Only. Not for Use in Diagnostic Procedures.

PRODUCT INFORMATION

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PRODUCT
The **K-*ASSAY*®** GST Fluorometric Activity Assay is for the quantitative determination of GST activity in plasma, cell and tissue homogenates. For research use only, not for diagnostic procedures.

PRINCIPLE
Glutathione S-transferase (GST) is a family of enzymes that play an important role in detoxification of xenobiotics. GST catalyzes the formation of the thiol group of glutathione to electrophilic xenobiotics. It utilizes glutathione to scavenge potentially toxic compounds including those produced as a result of oxidative stress and is part of the defense mechanism against the mutagenic, carcinogenic and toxic effects of such compounds. The **K-*ASSAY*®** GST Fluorometric Activity Assay provides a simple, fluorescence-based *in vitro* assay for detecting the GST activity using fluorescence plate reader. The assay utilizes monochlorobimane (MCB), a dye that reacts with glutathione. The free form of MCB is almost nonfluorescent, whereas the dye fluoresces blue (ex/em = 380/461 nm) when it reacts with glutathione. GST catalyzes the MCB-glutathione reactions and the fluorescence levels are proportional to the amounts of GST present in the reaction. Thus, the GST level in samples can be easily detected using a fluorometer of a 96-well fluorometric plate reader. The kit can detect GST activity in crude cell lysate or purified protein fraction and also quantitate GST-tagged fusion protein.

COMPONENTS
- GST Assay Buffer 10 mL
- GST Sample Buffer 25 mL
- MCB Substrate 200 µL
- Glutathione (lyophilized) 2 vials
- GST Calibrator (20 mU/µL) 20 µL

PROTOCOLS
Reagent Preparation and Storage
Glutathione: Add 550 µL of GST Sample Buffer to each vial just before use (200 mM). One vial is sufficient for 50 assays. Remaining solution can be kept at –20°C for 1 week.

GST Calibrator: Provided volume is sufficient for 10 calibration curves.

Sample Preparation Guideline
A. Cell Sample Preparation

1. Collect cells by centrifugation. For adherent cells, use a rubber policeman scraping the cells to collect.
2. Homogenize or sonicate the cells in GST Sample Buffer.
3. Centrifuge at 10,000 x g for 15 minutes at 4°C.
4. Collect supernatant and use for the assay. The remaining sample can be stored at –80°C, stable for at least 1 month.

B. Tissue Sample Preparation

1. Prior to dissection, perfuse tissue with PBS containing heparin (0.15 mg/mL) to remove red blood cells and clots.
2. Homogenize tissue in GST Sample Buffer (100 mg/0.5 mL).
3. Centrifuge at 10,000 x g for 15 minutes at 4°C.
4. Collect supernatant and use for the assay. The remaining sample can be stored at –80°C, stable for at least 1 month.

C. Plasma and Erythrocyte Sample Preparation

1. Centrifuge an anticoagulant treated blood at 1,000 x g for 10 minutes at 4°C.
2. Transfer the top plasma layer (without disturbing the white buffy layer) to a new tube and store on ice for assay or store at –80°C for future use. The plasma should be stable for 1 month.
3. Remove the white buffy layer and discard (leukocytes).
4. Lyse the erythrocytes (red blood cells) in 4 times its volume of ice-cold GST Sample Buffer.
5. Centrifuge at 10,000 x g for 15 minutes at 4°C.
6. Transfer supernatant (erythrocyte lysate) to a new tube, and use it for the GST assay. The remaining samples can be stored at –80°C for future use, stable for at least 1 month.

ASSAY PROTOCOL

1. Prepare sample in a total 100 µL volume with GST Sample Buffer, including a negative control with 100 µL of GST Sample Buffer only and a positive control with 2 µL of GST and 98 µL of Sample Buffer.
   Note: We recommend preparing several dilutions of your sample and performing duplicate wells for each measurement.
2. Add 10 µL of Glutathione to each well containing the sample or control above.
3. Prepare Substrate Mix by adding 2 µL MCB Solution to 98 µL of GST Assay Buffer for each sample including the calibrator. Mix well and transfer 100 µL of the mix into each sample and control well.
4. Carefully shake the plate to start the reaction.
5. Read samples at Ex./Em. 380/460 nm at various time points within 1 hour using a plate reader. At least 5 time points should be collected for kinetic studies. For general comparison, read samples in 0.5-1 hour to compare the fluorescence level of treated vs. control samples.
6. GST Activity can be expressed as RFU/min/mg protein. Alternatively, you may use the calibration curve to determine the GST activity in your samples.

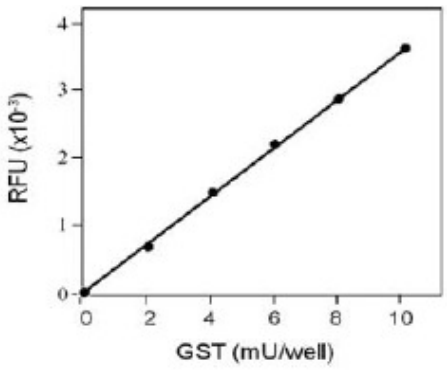

Figure 1: Calibration curve of GST measured by fluorometry. Various amounts of GST calibrator were incubated with GST and MCB according to the kit instructions. Fluorescence was measured at ex/em 380/460 nm.

STORAGE

Store at -20°C. The kit is stable until the expiration date shown on the label when stored at -20°C.

FOR RESEARCH USE ONLY

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