GST Fluorometric Activity Assay Kit

(Catalog #BN00528; 100 assays; Store kit at -20° C)

I. Introduction:

Glutathione S-transferase (GST) is a family of enzymes that plays an important role in the detoxification of xenobiotics by formation of glutathione adducts via the thiol group. GST utilizes glutathione to scavenge potentially toxic compounds including those produced as a result of oxidative stress, and is an important part of the defense mechanism against the mutagenic, carcinogenic and toxic effects of such compounds. The Assay Genie GST Fluorometric Activity Assay Kit provides a simple, fluorescence-based *in vitro* assay for detecting GST activity using a fluorescence plate reader. The assay utilizes monochlorobimane (MCB), a dye that reacts directly with glutathione. The free form of MCB is almost non-fluorescent, whereas the dye fluoresces blue (Ex/Em = 380/461 nm) after reaction with glutathione catalyzed by GST. The change in fluorescence over time is thus allows for an easy direct measurement of the sample GST level. The kit can detect GST activity in crude cell lysate or purified protein fraction. The kit can also be used to detect or quantitate GST-tagged fusion recombinant protein. Detection sensitivity < 0.5 mU.

II. Kit Contents:

Components	BN00528	Color Code	Part Number
GST Assay Buffer	25 ml	WM	BN00528-1
MCB Substrate (in DMSO)	200 µl	Red	BN00528-2
Glutathione (lyophilized)	2vials	Yellow	BN00528-3
GST Standard	10 µl	Green	BN00528-4

III. Reagent Preparation:

- **GST Assay Buffer:** Use as supplied. Store at 4°C or -20°C. Warm to room temperature before use.
- **MCB Substrate:** Warm to room temperature to thaw the DMSO solution before use. Store at -20°C.
- **Glutathione:** Add 550 µl of GST Assay Buffer to each vial just before use. Dissolve completely to generate 200 mM glutathione. One vial is sufficient for 50 assays. The remaining solution can be kept at –20°C for 1 week.
- **GST Standard**: Keep on ice while in use. Store at -80°C. Avoid multiple freeze/thaw cycles. Use within two months.

IV. Sample Preparation Guideline:

A. Cell Sample Preparation:

1. Collect cells by centrifugation. For adherent cells, use a rubber policeman or trypsinize to collect the cells.
2. Homogenize or sonicate the cells in 4 to 10 volume of Assay Buffer.
3. Centrifuge 10,000 x g for 15 minutes at 4°C and collect the supernatant. The supernatant can be stored at -80°C for at least one month for future experiments.

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 the tissue in 4 to 10 volume of Assay Buffer (e.g. homogenize 100 mg tissue with 0.5 ml GST Assay Buffer).
3. Centrifuge at 10,000 x g for 15 minutes at 4°C and collect the supernatant. The supernatant can be stored at -80°C for at least 1 month for future experiments.

C. Plasma and Erythrocyte Sample Preparation

1. Centrifuge anticoagulant treated blood samples at 1000 x g for 10 min at 4°C.
2. Transfer the top plasma layer (without disturbing the white buffy coat) to a new tube and store on ice for assay or store at -80°C for future use, stable for 1 month.
3. Remove the white buffy coat and discard (leukocytes).
4. Lyse the erythrocytes (red blood cells) in 4 volumes of ice-cold GST Assay Buffer.
4. Centrifuge at 10,000 x g for 15 min at 4°C. Transfer the supernatant (erythrocyte lysate) to a new tube, and use it for the GST assay. The supernatant can be stored at -80°C for at least 1 month for future experiments.

V. GST Activity Assay Protocol:

1. Prepare test samples in 96 well white plate. Adjust the final volume to 100 µl with GST Assay Buffer.
   Note: For unknown samples, we recommend preparing different doses of samples to make sure the readings are within the linear range.

2. **GST Standard Curve:** Dilute GST Standard 100 times by adding 2 µl of the GST Standard into 198 µl Assay Buffer to generate 1 mU/µl GST standard. Make just before use. Mix well. Add 0, 4, 8, 12, 16 and 20 µl of the 1 mU/µl standard into series of wells in 96 well plate to generate 0, 4, 8, 12, 16 and 20 mU/well of GST Standard. Adjust the final volume to 100 µl with Assay Buffer.

   Note: Discard the diluted GST Standard.

3. Add 10 µl of Glutathione to each well containing the samples and Standards.
4. **Reaction Mix:** Mix enough reagents for the number of assays to be performed. For each well, prepare 100 µl Mix containing:

	Reaction Mix
MCB Solution	2 µl
GST Assay Buffer	98 µl

Mix well. Add 100 µl of the Reaction Mix into each well containing samples and Standards. Mix the contents to start the reaction immediately.

5. **Measurement:** measure fluorescence at Ex/Em = 380/460 nm.
   Note: Incubation time depends on the GST activity in the samples. We recommend measuring fluorescence in a kinetic mode (every 5 min for 1 hour) and choose two time points (T_1 & T_2) in the linear range to calculate the GST activity of the samples.
6. **Calculation:** Subtract 0 Standard reading from all Standard readings. **Note:** 0 Standard reading could be significantly high. Calculate the GST activity of the test sample: $\Delta RFU = RFU_2 - RFU_1$. Apply the ΔRFU to the GST Standard Curve to get B mU of sample GST activity during the reaction time ($\Delta T = T_2 - T_1$).

Sample GST Activity = B/(ΔT X V) X Dilution Factor (mU/min/ml)

Where: **B** is sample GST activity from the GST Standard Curve (in mU)
ΔT is the reaction time (min).
V is the sample volume added into the reaction well (mL)

GSH molecular weight: 307.32 g/mol
GST molecular weight in the range of 22- 30 kDa.

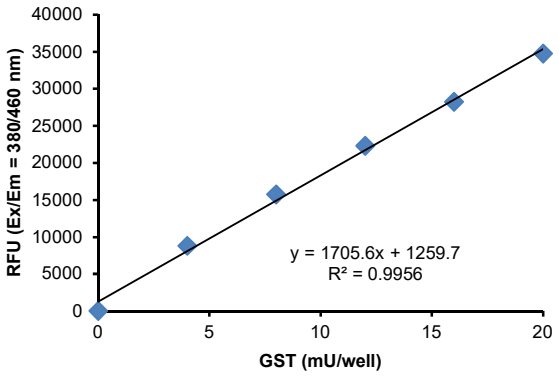

Fig. 1 Standard Calibration Curve of GST Measured by Fluorometry. Various amounts of Standard GST were incubated with GSH and MB according to the kit instructions. Fluorescence was measured at Ex/Em = 380/460 nm.

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