Product Information

GST Fluorometric Activity Assay Kit

I. Kit Contents:

Components	K2105-100 100 assays	Cap Color	Part Number
GST Assay Buffer	25 ml	WM	K2105-C-1
MCB Substrate (in DMSO)	200 µ	Red	K2105-C-2
Glutathione (lyophilized)	2 vial	Yellow	K2105-C-3
GST Standard	10 µ	Green	K2105-C-4

II. Introduction:

Glutathione S-transferase (GST) is a group of enzymes that involved in the detoxification of xenobiotics. GST catalyzes the formation of the thiol group of glutathione to electrophilic xenobitocs. It utilizes glutathione to scavenge toxic substances that produced by oxidative stress. The GST Fluorometric Activity Assay Kit 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 is nonfluorescent on its own, but emits blue (ex/em=380/461 nm) when reacts with glutathione. The MCB-glutathione reaction is catalyzed by GST and the fluorescence levels are proportionally to the amounts of GST in the reaction. Therefore, the GST level can be simply detected by a fluorometer or a 96-well fluorometric plate reader.

III. Reagent Preparation:

GST Assay Buffer: Use as supplied. Store at 4℃ or -20℃. Warm to room temperature before use.

MCB Substrate: Warm to room temperature to thaw the DMSO solution before use. Store at -20℃.

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℃ for 1 week.

GST Standard: Keep on ice while in use. Store at -80℃. 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℃ and collect the supernatant. The supernatant can be stored at -80℃ 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℃ and collect the supernatant. The supernatant can be stored at -80℃ 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℃.

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℃ 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.

5. Centrifuge at 10,000 x g for 15 min at 4℃. Transfer the supernatant (erythrocyte lysate) to a new tube, and use it for the GST assay. The supernatant can be stored at -80℃ 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.

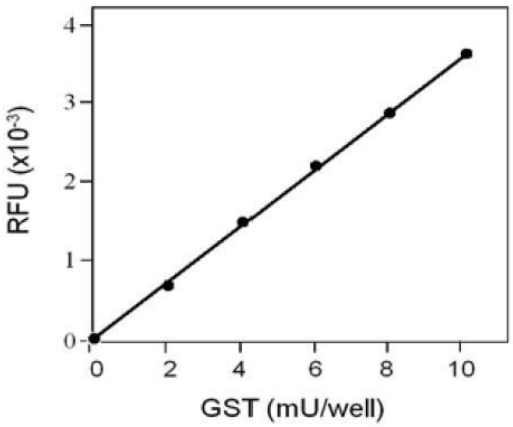

Fig. 1 Standard Calibration Curve of GST Measured by Fluorometry. Various amounts of Standard GST were incubated with GSH and MB according to the kit instructions. Fluorescence was measured at Ex/Em = 380/460 nm.

General Troubleshooting Guide:

Problems	Cause	Solution
Assay not working	• Use of a different buffer • Omission of a step in the protocol • Plate read at incorrect wavelength • Use of a different 96-well plate	• Refer data sheet and use buffers as indicated • Refer and follow the data sheet precisely • Check the wavelength in the data sheet and the filter settings of the instrument • Fluorescence: Black plates ; Luminescence: White plates; Colorimeters: Clear plates
Samples with erratic readings	• Use of an incompatible sample type • Samples prepared in a different buffer • Samples were not deproteinized (if indicated in datasheet) • Cell/ tissue samples were not completely homogenized • Lysates used after multiple free-thaw cycles • Presence of interfering substance in the sample • Use of old or inappropriately stored samples	• Refer data sheet for details about incompatible samples • Use the Nucleotide releasing buffer provided in the kit or refer data sheet for instructions • Use the 10 kDa spin cut-off filter or PCA precipitation as indicated • Use Dounce homogenizer (increase the number of strokes); observe for lysis under microscope • Aliquot and freeze samples if needed to use multiple times • Troubleshoot if needed, deproteinize samples • Use fresh samples or store at correct temperatures till use
Lower/ Higher readings in Samples and Standards	• Improperly thawed components • Use of expired kit or improperly stored reagents • Allowing the reagents to sit for extended times on ice • Incorrect incubation times or temperatures • Incorrect volumes used	• Thaw all components completely and mix gently before use • Always check the expiry date and store the components appropriately • Always thaw and prepare fresh reaction mix before use • Refer data sheet & verify correct incubation times and temperatures • Use calibrated pipettes and aliquot correctly
Unanticipated results	• Measured at incorrect wavelength • Samples contain interfering substances • Use of incompatible sample type	• Check the equipment and the filter setting • Troubleshoot if it interferes with the kit • Refer data sheet to check if sample is compatible with the kit

	• Sample readings above/below the linear range	or optimization is needed • Concentrate/ Dilute sample so as to be in the linear range
Note: The most probable list of causes is under each problem section. Causes/ Solutions may overlap with other problems.		

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Our promise

If the product does not perform as described on this datasheet, we will offer a refund or replacement. For more details, please visit http://www.apexbt.com/ or contact our technical team.

Tel: +1-(832)696-8203
Fax: +1-832-641-3177
Email: sales@apexbt.com