Product Information

GST Colorimetric Activity Assay Kit

I. Kit Contents:

Components	K2108-100 100 assays	Cap Color	Part Number
GST Assay Buffer	25 ml	WM	K2108-C-1
GST Substrate (CDNB)	0.1 ml	Red	K2108-C-2
Glutathione (GSH, lyophilized)	2 x 17 mg	Yellow	K2108-C-3
GST Positive Control	10 µl	Green	K2108-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 Colorimetric Activity Assay Kit is rely on the GST-catalyzed reaction of GSH and the GST substrate-CDNB (1-chloro-2,4-dinitrobenzene). A dinitrophenyl thioether is produced by GST-catalyzed formation of GS-DNB. It can be detected by spectrophotometer at 340 nm. The kit can detect GST activity in crude cell lysate, purified protein fraction and quantitate GST-tagged fusion protein.

III. Reagent Preparation and Storage Conditions:

GST Assay Buffer: store at 4℃

GSH: Add 275 µl of GST Assay Buffer to each vial just before use. One vial is sufficient for 50 assays. The Remaining solution can be kept at -20℃ for 1 week.

CDNB: This vial contains a DMSO solution of 1-chloro-2, 4-dinitrobenzene (CDNB) and should be stored at -20℃.

GST Positive Control: Store at -20℃

IV. Sample Preparation Guideline:

A. Cell Sample Preparation:

1. Collect cells by centrifugation. For adherent cells, use a rubber policeman to scrape and collect the cells.
2. Homogenize or sonicate the cells in GST Assay Buffer (typically 3 -4 volumes).
3. Centrifuge at 10,000 x g for 15 min at 4℃.
4. Collect supernatant and use for the assay. The remaining sample should be stored at -80℃, and is 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 Assay Buffer (100 mg/0.5 ml).
3. Centrifuge at 10,000 x g for 15 minutes at 4℃.
4. Collect supernatant and use for the assay. The remaining sample should be stored at -80℃, and is stable for at least 1 month.

C. Plasma and Erythrocyte Sample Preparation:

1. Centrifuge anticoagulant treated blood at 1000 x g for 10 min at 4℃.

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℃ 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 Assay Buffer.

5. Centrifuge at 10,000 x g for 15 min at 4℃.

6. Transfer supernatant (erythrocyte lysate) to a new tube, and use it for the GST assay. The remaining samples should be stored at -80℃ for future use and is stable for at least one month.

D. Preparation of Bacterially Expressed GST-Fusion Protein Sample:

1. Collect bacteria by centrifugation. Freeze/thaw the pellet two times, then sonicate in GST Assay Buffer.

2. Centrifuge at 10,000 x g for 15 min at 4℃.

3. Transfer supernatant to a new tube, and use it for the GST assay. The remaining samples should be stored at -80℃ for future use and is stable for at least one month.

V. GST Assay Protocol:

1. Sample, Negative Control and Positive Control Preparation: Prepare samples in a total 50 µl volume with GST Assay Buffer, including a negative control with 50 µl of GST Assay buffer only. For GST Positive Control, dilute 100 time by adding 2 µl of Positive Control into 198 µl GST Assay Buffer, add 2-10 µl of diluted GST Positive Control into desired well (s) and adjust the final volume to 50 µl with GST Assay Buffer.

Note: We recommend preparing several dilutions of your sample and running duplicate wells for each measurement.

2. Glutathione Addition: Add 5 µl of Glutathione to each well containing the sample or control above.

3. Substrate Mix: Mix enough reagents for the number of assays to be performed. For each well, prepare a total 50 µl Substrate Mix containing:

GST Assay Buffer 49 µl

GST Substrate (CDNB) Solution 1 µl

Mix well and transfer 50 µl of the Mix into each sample (including the standard) well.

4. Measurement: Carefully shake the plate to start the reaction. Read the absorbance once every minute at 340 nm using a plate reader to obtain at least 5 time points. For low GST activity samples, the reaction can be continued for longer time periods.

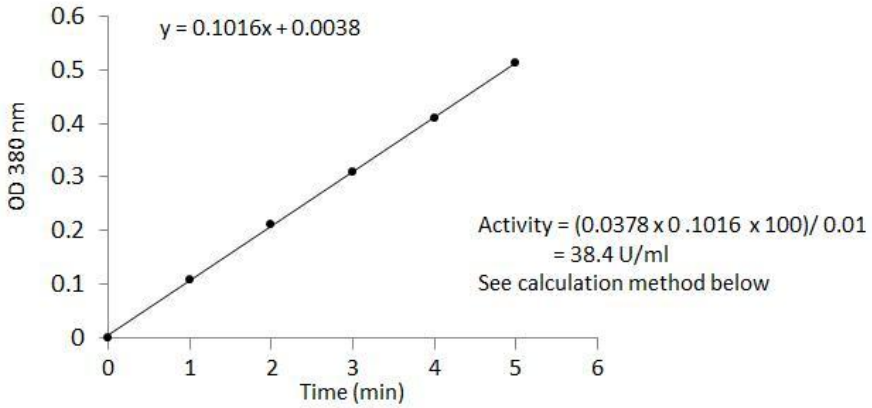

Figure 1: GST Kinetic Assay Performed According to This Protocol

5. Calculation of GST Assay Results:

a) Determine the change in absorbance (ΔA340) per minute by:

i. Plotting the absorbance values as a function of time to obtain the slope (rate) of the linear portion of the curve.

ii. Select two points on the linear portion of the curve and determine the change in absorbance during that time, using the following equation:

$\Delta A340/min = [A340\ (Time_2) - A340\ (Time_1)]/ [Time_2\ (min) - Time_1\ (min)]$

b) Determine the rate of ΔA340/min for the background wells and subtract the rate from that of the sample wells.

c) Use the following formula to calculate the GST activity (U/ml of sample). The reaction rate at 340 nm can be determined using the GS-DNB extinction coefficient at 340 nm 0.0096 $\mu M^{-1}cm^{-1}$. The value has been adjusted for the path length of the solution in the well 0.2893 cm).

GST Activity = $(\Delta A340min^{-1} \times$ Reaction Volume (ml)$)/(0.0096\ \mu mol^{-1}cm^{-1} \times 1000\ ml \times 0.2893\ cm \times V) \times D$

$= \Delta A340min^{-1}/ \times 0.036 \times D/V$ (μmol/min/ml)

Where:

$0.0096\ \mu mol^{-1}cm^{-1}$ is the extinction coefficient of the glutathione-DNB adduct.

V = Sample Volume added to well (ml).

D = Sample Dilution Factor.

0.2893 cm is light path of the 0.1 ml Reaction Volume in a Greiner Bio One.

655101 96 well plate (cm). Other plates must be calibrated for accurate results.

Unit Definition: One unit is the amount of enzyme that conjugates 1.0 μmol of 1- Chloro-2,4-Dinitrobenzene with reduced glutathione per min. at pH 6.4 at 25℃.

General Troubleshooting Guide:

Problems	Cause	Solution
Assay not working	• Use of ice-cold assay buffer • Omission of a step in the protocol • Plate read at incorrect wavelength • Use of a different 96-well plate	• Assay buffer must be at room temperature • 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 • Cell/ tissue samples were not completely homogenized • Samples 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 reaction buffer provided in the kit or refer data sheet for instructions • 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
Readings do not follow a linear pattern for	• Use of partially thawed components • Pipetting errors in the standard • Pipetting errors in the reaction mix	• Thaw and re-suspend all components before preparing the reaction mix • Avoid pipetting small volumes

Standard curve	• Air bubbles formed in well • Standard stock is at an incorrect concentration • Calculation errors • Substituting reagents from older kits/ lots	• Pipette gently against the wall of the tubes • Prepare a master reaction mix whenever possible • Always refer the dilutions in the data sheet • Use fresh components from the same kit • Recheck calculations after referring the data sheet
Unanticipated results	• Measured at incorrect wavelength • Samples contain interfering substances • Use of incompatible sample type • Sample readings above/below the linear range	• 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 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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