GST Colorimetric Activity Assay Kit

Introduction

Glutathione S-transferases (GSTs) are a group of enzymes that are 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 relies 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 microplate reader at 340 nm.

Components and Storage

Components \ Size	100 Assays	Storage
GST Assay Buffer	25 mL	-20°C
CDNB	100 µL	-20°C away from light
GSH	2 X 17 mg	-20°C
GST Positive Control (20X)	10 µL	-20°C
Shipping: Blue ice	shelf life: 1 year	

Protocol

1. **Reagent Preparation:**

   1) GST Assay Buffer: Warm to room temperature before use.

   2) CDNB: Place in a 37°C bath for 1-5 min to thaw the solution before use. Then keep it on ice while in use. Once thawed, aliquot it and use within two months.

   3) 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°C for 1 week.

   4) GST Positive Control (20X): Dilute 10 µL GST Positive Control (20X) in 190 µL GST Assay Buffer to make the GST Positive Control (1X).

2. **Sample Preparation Guideline:**

   A. Cell Sample Preparation:

      1) Collect cells by centrifugation (initial recommendation = 2 x 10^6 cells). Wash cells with PBS and

resuspend in 100 µL of GST Assay Buffer.

2) Homogenize or sonicate the cells in GST Assay Buffer.

3) Centrifuge at 4°C, 10000 x g for 15 min.

4) Transfer supernatant to a new tube and keep on ice for use.

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) Wash tissue in PBS. Then homogenize tissue on ice in GST Assay Buffer (100 mg/0.5 mL).

3) Centrifuge at 4°C, 10000 x g for 15 min.

4) Transfer supernatant to a new tube and keep on ice for use.

C. Plasma and Erythrocyte Sample Preparation:

1) Centrifuge anticoagulant treated blood at 1000 x g for 10 min 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 volume of ice-cold GST Assay Buffer.

5) Centrifuge at 4°C, 10000 x g for 15 min.

6) Transfer supernatant (erythrocyte lysate) to a new tube, and keep on ice for use. The remaining samples should be stored at -80°C 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 4°C, 10000 x g for 15 min.

3) Transfer supernatant to a new tube and keep on ice for use. The remaining samples should be stored at -80°C for future use and is stable for at least one month.

3. GST Assay Protocol:

1) Set up reaction wells

Sample wells	5-50 µL sample (adjust volume to 50 µL with GST Assay Buffer)

Positive wells	2-10 µL GST Positive Control (1X) (adjust volume to 50 µL with GST Assay Buffer)
Background wells	50 µL GST Assay Buffer

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

2) Add 5 µl of GSH to each well.

3) Prepare 50 µL of reaction mix for each reaction. Mix enough reagents for the number of assays to be performed.

Reagents	**Volume**
GST Assay Buffer	49 µL
CDNB	1 µL

4) Mix well and transfer 50 µL of the reaction mix into each well.

5) Carefully shake the plate to start the reaction. Read the absorbance once every 2-3 min at 340 nm using a plate reader for at least 10 min at room temperature away from light. For low GST activity samples, the reaction can be continued for longer periods.

6) 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:

ΔA340/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 = ($\Delta A340min^{-1}$×Reaction Volume (mL))/ (0.0096 $\mu mol^{-1}cm^{-1}$ × 1000 mL × 0.2893 cm × V) × D = $\Delta A340min^{-1}$x 0.036 x 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°C.

Troubleshooting

Problem	Cause	Solution
Assay not working	Use of ice-cold buffer	Buffers must be at room temperature
	Plate read at incorrect wavelength	Check the wavelength and filter settings of instrument
	Use of a different 96-well plate	Colorimetric: Clear plates Fluorometric: black wells/clear bottom plate
Sample with erratic readings	Samples not deproteinized (if indicated on protocol)	Use provided protocol for deproteinization
	Cells/tissue samples not homogenized completely	Use Dounce homogenizer, increase number of strokes
	Samples used after multiple free/thaw cycles	Aliquot and freeze samples if needed to use multiple times
	Use of old or inappropriately stored samples	Use fresh samples or store at -80°C (after snap freeze in liquid nitrogen) till use
	Presence of interfering substance in the sample	Check protocol for interfering substances; deproteinize samples
Lower/Higher readings in samples and Standards	Improperly thawed components	Thaw all components completely and mix gently before use
	Allowing reagents to sit for extended times on ice	Always thaw and prepare fresh reaction mix before use
	Incorrect incubation times or temperatures	Verify correct incubation times and temperatures in protocol
Standard readings do not follow a linear pattern	Pipetting errors in standard or reaction mix	Avoid pipetting small volumes (< 5 μL) and prepare a master mix whenever possible
	Air bubbles formed in well	Pipette gently against the wall of the tubes
	Standard stock is at incorrect concentration	Always refer to dilutions described in the protocol
Unanticipated results	Measured at incorrect wavelength	Check equipment and filter setting
	Samples contain interfering substances	Troubleshoot if it interferes with the kit
	Sample readings above/ below the linear range	Concentrate/ Dilute sample so it is within the linear range

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