ab65325

GST Activity Assay Kit (Fluorometric)

Instructions for Use

For the rapid, sensitive and accurate measurement of GST Activity in various samples

This product is for research use only and is not intended for diagnostic use.

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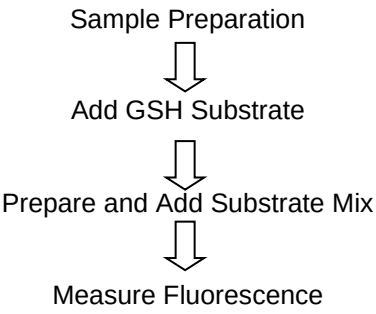
1. Overview

Glutathione S-transferase (GST) is a family of enzymes that play an important role in 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.

Abcam's GST Activity Assay Kit (Fluorometric) 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 in blue (Ex/Em = 380/461 nm) after reaction with glutathione catalysed by GST. The change in fluorescence overtime allows for an easy measurement of the sample GST level.

The kit can detect GST activity in crude cell lysate or purified protein fraction, and also quantitate GST-tagged fusion protein. Detects <0.5 mU.

2. Protocol Summary



3. Components and Storage

A. Kit Components

Item	Quantity
GST Assay Buffer	25 mL
MCB Substrate	200 µL
GSH Substrate	2 x vial
GST Enzyme Standard	10 µL

* Store kit at -20°C.Read the entire protocol before performing the assay.

GST ASSAY BUFFER: Use as supplied. Store at -20°C. Allow to warm to room temperature before use.

MCB SUBSTRATE: Warm to room temperature to thaw the DMSO solution before use. Store at -20°C.

GSH Substrate: Add 550 µL of GST Assay Buffer to each vial just before use. Dissolve completely to generate 200 mM GSH Substrate. One vial is sufficient for 50 assays. The remaining solution can be kept at -20°C for 1 week.

GST Enzyme Standard: Keep on ice while in use. Store at -80°C. Avoid multiple freeze/thaw cycles. Use within two months.

B. Additional Materials Required

- Microcentrifuge
- Pipettes and pipette tips
- Fluorescent microplate reader
- 96 well plate
- Orbital shaker

4. Assay Protocol

1. Sample Preparation:

a. For cell samples:

i. Collect cells by centrifugation. For adherent cells, use a rubber policeman to collect the cells.

ii. Homogenize or sonicate the cells in 4 to 10 volume of GST Assay Buffer. Centrifuge 10,000 x g for 15 minutes at +4°C and collect the supernatant.

The supernatant should be stored at -80°C, stable for at least 1 month.

b. For tissue samples:

i. Prior to dissection, perfuse tissue with PBS containing heparin (0.15 mg/mL) to remove red blood cells and clots.

ii. Homogenize tissue in 4 to 10 volume of GST Assay Buffer (100 mg/0.5 mL).

iii. Centrifuge at 10,000 x g for 15 minutes at +4°C. Collect supernatant and use for the assay.

The remaining pellet should be stored at -80°C, and is stable for at least 1 month

c. For plasma and erythrocyte samples:

i. Centrifuge anticoagulant treated blood at 1000 x g for 10 min at +4°C.

ii. Transfer the top plasma layer (without disturbing the white buffer 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.

iii. Remove the white buffy layer and discard (leukocytes). Lyse the erythrocytes (red blood cells) in 4 times its volume of ice-cold GST Assay Buffer.

iv. Centrifuge at 10,000 x g for 15 min at +4°C.

v. Transfer supernatant (erythrocyte lysate) to a new tube, and use it for the GST assay.

The remaining samples should be stored at -80°C for future use and is stable for at least one month.

2. Prepare test samples in 96 well plate. Adjust the final volume to 100 µL with GST Assay Buffer.

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

3. **GST Standard Curve:** Dilute GST Enzyme Standard 100 times by adding 2 µL of the GST Enzyme Standard into 198 µL Assay Buffer to generate 1 mU/µL GST Enzyme Standard. 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 Enzyme Standard. Adjust the final volume to 100 µL with GST Assay Buffer.

   Note: Discard the diluted GST Enzyme Standard.

4. **GSH Substrate:**

   Add 10 µL of GSH Substrate to each well containing the sample and Standards.

5. **Substrate Mix:**

   Mix enough reagents for the number of assays to be performed. For each well, prepare a total 100 µL Substrate Mix containing:

GST Assay Buffer	98 µL
MCB Solution	2 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.

6. 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.

5. Data Analysis

Subtract zero Standard reading from all Standard readings.

NOTE: Zero 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:

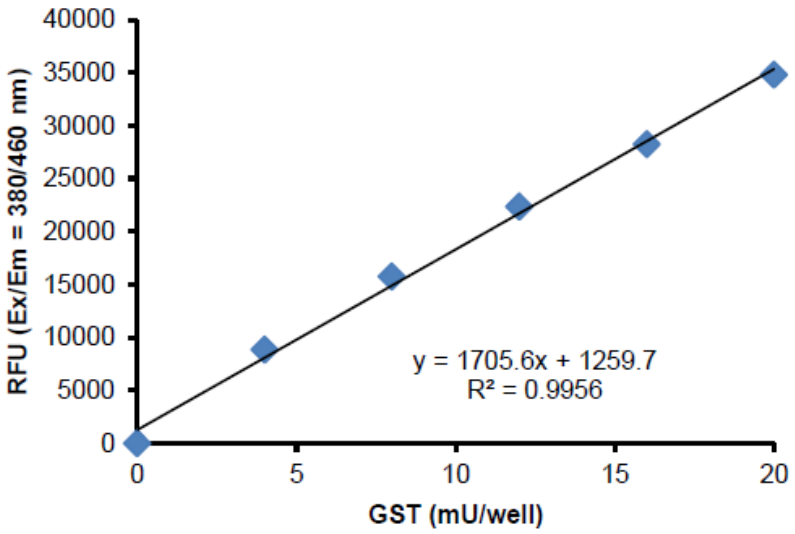
B is sample GST activity from the GST Standard Curve (in mU)

ΔT is the reaction time (min).

V is the sample volume added into the reaction well (in mL)

GSH molecular weight: 307.32 g/mol

GST molecular weight is in the range of 22- 30 kDa.



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

6. Troubleshooting

Problem	Reason	Solution
Assay not working	Assay buffer at wrong temperature	Assay buffer must not be chilled - needs to be at RT
	Protocol step missed	Re-read and follow the protocol exactly
	Plate read at incorrect wavelength	Ensure you are using appropriate reader and filter settings (refer to datasheet)
	Unsuitable microtiter plate for assay	Fluorescence: Black plates (clear bottoms); Luminescence: White plates; Colorimetry: Clear plates. If critical, datasheet will indicate whether to use flat- or U-shaped wells
Unexpected results	Measured at wrong wavelength	Use appropriate reader and filter settings described in datasheet
	Samples contain impeding substances	Troubleshoot and also consider deproteinizing samples
	Unsuitable sample type	Use recommended samples types as listed on the datasheet
	Sample readings are outside linear range	Concentrate/ dilute samples to be in linear range

Samples with inconsistent readings	Unsuitable sample type	Refer to datasheet for details about incompatible samples
	Samples prepared in the wrong buffer	Use the assay buffer provided (or refer to datasheet for instructions)
	Samples not deproteinized (if indicated on datasheet)	Use the ***10kDa spin column (ab93349)***
	Cell/ tissue samples not sufficiently homogenized	Increase sonication time/ number of strokes with the Dounce homogenizer
	Too many freeze-thaw cycles	Aliquot samples to reduce the number of freeze-thaw cycles
	Samples contain impeding substances	Troubleshoot and also consider deproteinizing samples
	Samples are too old or incorrectly stored	Use freshly made samples and store at recommended temperature until use
Lower/ Higher readings in samples and standards	Not fully thawed kit components	Wait for components to thaw completely and gently mix prior use
	Out-of-date kit or incorrectly stored reagents	Always check expiry date and store kit components as recommended on the datasheet
	Reagents sitting for extended periods on ice	Try to prepare a fresh reaction mix prior to each use
	Incorrect incubation time/ temperature	Refer to datasheet for recommended incubation time and/ or temperature
	Incorrect amounts used	Check pipette is calibrated correctly (always use smallest volume pipette that can pipette entire volume)

Problem	Reason	Solution
Standard curve is not linear	Not fully thawed kit components	Wait for components to thaw completely and gently mix prior use
	Pipetting errors when setting up the standard curve	Try not to pipette too small volumes
	Incorrect pipetting when preparing the reaction mix	Always prepare a master mix
	Air bubbles in wells	Air bubbles will interfere with readings; try to avoid producing air bubbles and always remove bubbles prior to reading plates
	Concentration of standard stock incorrect	Recheck datasheet for recommended concentrations of standard stocks
	Errors in standard curve calculations	Refer to datasheet and re-check the calculations
	Use of other reagents than those provided with the kit	Use fresh components from the same kit

Technical Support

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