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

Sample Preparation
   ↓
Add Glutathione
   ↓
Prepare and Add Substrate Mix
   ↓
Measure Fluorescence
3. Components and Storage

A. Kit Components

<table>
<thead>
<tr>
<th>Item</th>
<th>Quantity</th>
</tr>
</thead>
<tbody>
<tr>
<td>GST Assay Buffer</td>
<td>25 mL</td>
</tr>
<tr>
<td>MCB Substrate (in DMSO)</td>
<td>200 µL</td>
</tr>
<tr>
<td>Glutathione (Lyophilized)</td>
<td>2 x vial</td>
</tr>
<tr>
<td>GST Standard</td>
<td>10 µL</td>
</tr>
</tbody>
</table>

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

GST ASSAY BUFFER: Use as supplied. Store at 4°C or -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.

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.

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

   **Note:** Discard the diluted GST Standard.

4. **Glutathione Addition:**
   Add 10 µL of Glutathione 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
   - MCB Solution
   
   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 (T₁ & T₂) 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: \( \Delta \text{RFU} = \text{RFU}_2 - \text{RFU}_1 \).

Apply the \( \Delta \text{RFU} \) to the GST Standard Curve to get B mU of sample GST activity during the reaction time (\( \Delta T = T_2 - T_1 \)).

\[
\text{Sample GST Activity} = \frac{B}{(\Delta T \times V)} \times \text{Dilution Factor (mU/min/mL)}
\]

Where:

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

\( \Delta T \) 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

<table>
<thead>
<tr>
<th>Problem</th>
<th>Reason</th>
<th>Solution</th>
</tr>
</thead>
<tbody>
<tr>
<td>Assay not working</td>
<td>Assay buffer at wrong temperature</td>
<td>Assay buffer must not be chilled - needs to be at RT</td>
</tr>
<tr>
<td>Protocol step missed</td>
<td>Re-read and follow the protocol exactly</td>
<td></td>
</tr>
<tr>
<td>Plate read at incorrect wavelength</td>
<td></td>
<td>Ensure you are using appropriate reader and filter settings (refer to datasheet)</td>
</tr>
<tr>
<td>Unsuitable microtiter plate for assay</td>
<td></td>
<td>Fluorescence: Black plates (clear bottoms); Luminescence: White plates; Colorimetry: Clear plates. If critical, datasheet will indicate whether to use flat- or U-shaped wells</td>
</tr>
<tr>
<td>Unexpected results</td>
<td>Measured at wrong wavelength</td>
<td>Use appropriate reader and filter settings described in datasheet</td>
</tr>
<tr>
<td>Samples contain impeding substances</td>
<td></td>
<td>Troubleshoot and also consider deproteinizing samples</td>
</tr>
<tr>
<td>Unsuitable sample type</td>
<td></td>
<td>Use recommended samples types as listed on the datasheet</td>
</tr>
<tr>
<td>Sample readings are outside linear range</td>
<td></td>
<td>Concentrate/ dilute samples to be in linear range</td>
</tr>
<tr>
<td>Samples with inconsistent readings</td>
<td>Unsuitable sample type</td>
<td>Refer to datasheet for details about incompatible samples</td>
</tr>
<tr>
<td>-----------------------------------</td>
<td>------------------------</td>
<td>----------------------------------------------------------</td>
</tr>
<tr>
<td>Samples prepared in the wrong buffer</td>
<td>Use the assay buffer provided (or refer to datasheet for instructions)</td>
<td></td>
</tr>
<tr>
<td>Samples not deproteinized (if indicated on datasheet)</td>
<td>Use the <strong>10kDa spin column (ab93349)</strong></td>
<td></td>
</tr>
<tr>
<td>Cell/ tissue samples not sufficiently homogenized</td>
<td>Increase sonication time/ number of strokes with the Dounce homogenizer</td>
<td></td>
</tr>
<tr>
<td>Too many freeze-thaw cycles</td>
<td>Aliquot samples to reduce the number of freeze-thaw cycles</td>
<td></td>
</tr>
<tr>
<td>Samples contain impeding substances</td>
<td>Troubleshoot and also consider deproteinizing samples</td>
<td></td>
</tr>
<tr>
<td>Samples are too old or incorrectly stored</td>
<td>Use freshly made samples and store at recommended temperature until use</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Lower/ Higher readings in samples and standards</th>
<th>Not fully thawed kit components</th>
<th>Wait for components to thaw completely and gently mix prior use</th>
</tr>
</thead>
<tbody>
<tr>
<td>Out-of-date kit or incorrectly stored reagents</td>
<td>Always check expiry date and store kit components as recommended on the datasheet</td>
<td></td>
</tr>
<tr>
<td>Reagents sitting for extended periods on ice</td>
<td>Try to prepare a fresh reaction mix prior to each use</td>
<td></td>
</tr>
<tr>
<td>Incorrect incubation time/ temperature</td>
<td>Refer to datasheet for recommended incubation time and/ or temperature</td>
<td></td>
</tr>
<tr>
<td>Incorrect amounts used</td>
<td>Check pipette is calibrated correctly (always use smallest volume pipette that can pipette entire volume)</td>
<td></td>
</tr>
<tr>
<td>Problem</td>
<td>Reason</td>
<td>Solution</td>
</tr>
<tr>
<td>-------------------------------</td>
<td>---------------------------------------------</td>
<td>--------------------------------------------------------------------------</td>
</tr>
<tr>
<td>Standard curve is not linear</td>
<td>Not fully thawed kit components</td>
<td>Wait for components to thaw completely and gently mix prior use</td>
</tr>
<tr>
<td></td>
<td>Pipetting errors when setting up the standard curve</td>
<td>Try not to pipette too small volumes</td>
</tr>
<tr>
<td></td>
<td>Incorrect pipetting when preparing the reaction mix</td>
<td>Always prepare a master mix</td>
</tr>
<tr>
<td></td>
<td>Air bubbles in wells</td>
<td>Air bubbles will interfere with readings; try to avoid producing air bubbles and always remove bubbles prior to reading plates</td>
</tr>
<tr>
<td></td>
<td>Concentration of standard stock incorrect</td>
<td>Recheck datasheet for recommended concentrations of standard stocks</td>
</tr>
<tr>
<td></td>
<td>Errors in standard curve calculations</td>
<td>Refer to datasheet and re-check the calculations</td>
</tr>
<tr>
<td></td>
<td>Use of other reagents than those provided with the kit</td>
<td>Use fresh components from the same kit</td>
</tr>
</tbody>
</table>
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