ab65326

GST Activity Assay Kit
(Colorimetric)

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.
ab65327 Nitric Oxide Assay Kit (Fluorometric)
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1. Overview

Glutathione S-transferase (GST) is a family of enzymes that play an important role in detoxification of xenobiotics. GST catalyzes the formation of the thiol group of glutathione to electrophilic xenobiotics. It utilizes glutathione to scavenge potentially toxic compounds including those produced as a result of oxidative stress and is part of the defense mechanism against the mutagenic, carcinogenic and toxic effects of such compounds.

Abcam’s GST Activity Assay Kit (Colorimetric) is based upon the GST-catalyzed reaction between GSH and the GST substrate, CDNB (1-chloro-2,4-dinitrobenzene, which has the broadest range of isozyme detectability (e.g., alpha-, mu-, pi-, and other GST isoforms). Under certain conditions, the interaction between glutathione and CDNB is totally dependent on the presence of active GST.

\[
\text{GSH} + \text{Cl} \quad \text{NO}_2 \quad \xrightarrow{\text{GST}} \quad \text{GS} \quad \text{NO}_2 + \text{Cl}^- + \text{H}^+
\]

The GST-catalyzed formation of GS-DNB produces a dinitrophenyl thioether which can be detected by spectrophotometer at 340 nm. One unit of GST activity is defined as the amount of enzyme producing 1 µmol of GS-DNB conjugate/min under the conditions of the assay. The kit can detect GST activity in crude cell lysate or
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purified protein fraction, and also quantitate GST-tagged fusion protein. Detect limit: Active GST < 4 mU.

2. Protocol Summary

Sample Preparation

\[\downarrow\]

Add Glutathione

\[\downarrow\]

Prepare and Add Substrate Mix

\[\downarrow\]

Measure Absorbance
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>GST Substrate (CDNB)</td>
<td>0.5 mL</td>
</tr>
<tr>
<td>Glutathione (GSH, Lyophilized)</td>
<td>2 x vial</td>
</tr>
<tr>
<td>GST Positive Control (0.625 μg/μl)</td>
<td>20 μL</td>
</tr>
</tbody>
</table>

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

GST ASSAY BUFFER: May be stored at +4 °C

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.

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

GST Positive Control: Store at -20 °C
B. Additional Materials Required

- Microcentrifuge
- Pipettes and pipette tips
- Colorimetric microplate reader
- 96 well plate
- Orbital shaker
4. Assay Protocol

1. Sample Preparation:

a. For cell samples:
   a) Collect cells by centrifugation. For adherent cells, use a rubber policeman to scrape and collect the cells.
   b) Homogenize or sonicate the cells in GST Assay Buffer (typically 3-4 volumes).
   c) Centrifuge at 10,000 x g for 15 min at +4°C.
   d) Collect supernatant and use for the assay. The remaining sample should be stored at -80°C, and is stable for at least 1 month.

b. For tissue samples:
   a) Prior to dissection, perfuse tissue with PBS containing heparin (0.15 mg/ml) to remove red blood cells and clots.
   b) Homogenize tissue in GST Assay Buffer (100 mg/0.5 ml).
   c) Centrifuge at 10,000 x g for 15 minutes at +4°C.
   d) Collect supernatant and use for the assay. The remaining sample should be stored at -80°C, and is stable for at least 1 month.
c. **For plasma and erythrocyte samples:**
   a) Centrifuge anticoagulant treated blood at 1000 x g for 10 min at +4°C.
   b) 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.
   c) Remove the white buffy layer and discard (leukocytes).
   d) Lyse the erythrocytes (red blood cells) in 4 times its volume of ice-cold GST Assay Buffer.
   e) Centrifuge at 10,000 x g for 15 min at +4°C.
   f) 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.

d. **For bacterially expressed GST-fusion proteins:**
   a) Collect bacteria by centrifugation. Freeze/thaw the pellet two times, then sonicate in GST Assay Buffer.
   b) Centrifuge at 10,000 x g for 15 min at +4°C.
   c) Transfer supernatant 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.
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Prepare samples in a total 50 μl volume with GST Assay Buffer, including a negative control with 50 μl of GST Assay buffer only and a positive control (10 μl of GST positive control diluted 1:50) and 40 μl of GST Assay Buffer.

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

- GST Assay Buffer 45 μl
- GST Substrate (CDNB) Solution 5 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.
5. Data Analysis

1. Determine the change in absorbance ($\Delta A_{340}$) per minute by:
   a) Plotting the absorbance values as a function of time to obtain the slope (rate) of the linear portion of the curve.

   b) Selecting two points on the linear portion of the curve and determining the change in absorbance during that time, using the following equation:

   \[
   \frac{A_{340} \text{ (Time 2)} - A_{340} \text{ (Time 1)}}{\text{Time 2 (min) - Time 1 (min)}}
   \]

2. Determine the rate of $\Delta A_{340}/\text{min}$ for the background wells and subtract the rate from that of the sample wells.

3. 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 μM$^{-1}$cm$^{-1}$. The value has been adjusted for the path length of the solution in the well 0.2893 cm.

   \[
   \text{GST Activity} = \frac{A_{340 \text{ min}^{-1}} \times \text{Reaction Volume (ml)}}{0.0096 \ \mu\text{mol}^{-1}\text{cm}^{-1} \times 1000 \ \text{ml} \times 0.2893 \ \text{cm} \times A} = \frac{\Delta A_{340 \text{ min}^{-1}}}{0.002777 \mu\text{mol} \times D/A} \ (\mu\text{mol/minute/ml})
   \]

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

A is the Sample Volume added to well (ml)

D is the 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.

GST Kinetic Assay Performed According to This Protocol
## 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></td>
<td>Re-read and follow the protocol exactly</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>
</tbody>
</table>
### Samples with inconsistent readings

<table>
<thead>
<tr>
<th>Problem</th>
<th>Reason</th>
<th>Solution</th>
</tr>
</thead>
<tbody>
<tr>
<td>Unsuitable sample type</td>
<td>Refer to datasheet for details about incompatible samples</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 10kDa spin column (ab93349)</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>

### Lower/ Higher readings in samples and standards

<table>
<thead>
<tr>
<th>Problem</th>
<th>Reason</th>
<th>Solution</th>
</tr>
</thead>
<tbody>
<tr>
<td>Not fully thawed kit components</td>
<td>Wait for components to thaw completely and gently mix prior use</td>
<td></td>
</tr>
<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>
</tbody>
</table>
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<table>
<thead>
<tr>
<th>Standard curve is not linear</th>
<th>Not fully thawed kit components</th>
<th>Wait for components to thaw completely and gently mix prior to use</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pipetting errors when setting up the standard curve</td>
<td>Try not to pipette too small volumes</td>
<td></td>
</tr>
<tr>
<td>Incorrect pipetting when preparing the reaction mix</td>
<td>Always prepare a master mix</td>
<td></td>
</tr>
<tr>
<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>
<td></td>
</tr>
<tr>
<td>Concentration of standard stock incorrect</td>
<td>Recheck datasheet for recommended concentrations of standard stocks</td>
<td></td>
</tr>
<tr>
<td>Errors in standard curve calculations</td>
<td>Refer to datasheet and re-check the calculations</td>
<td></td>
</tr>
<tr>
<td>Use of other reagents than those provided with the kit</td>
<td>Use fresh components from the same kit</td>
<td></td>
</tr>
</tbody>
</table>

For further technical questions please do not hesitate to contact us by email (technical@abcam.com) or phone (select “contact us” on www.abcam.com for the phone number for your region).
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