ab65326
GST Activity Assay Kit (Colorimetric)

Instructions for use:

For quantitative measurement of glutathione-S-transferase (GST) activity in biological samples.

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

PLEASE NOTE: With the acquisition of BioVision by Abcam, we have made some changes to component names and packaging to better align with our global standards as we work towards environmental-friendly and efficient growth. You are receiving the same high-quality products as always, with no changes to specifications or protocols.

Version 16b Last Updated 23 April 2025

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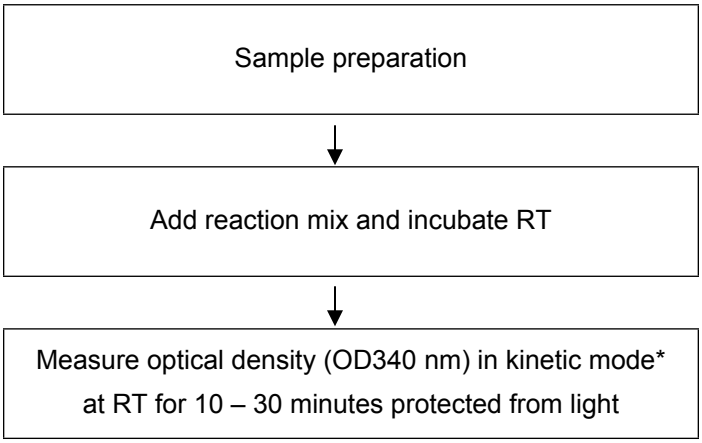
1. BACKGROUND

GST Activity Assay Kit (Colorimetric) (ab65326) is a simple and accurate to test to measure activity of Glutathione-S-transferase (GST) in a variety of biological samples. The kit is based upon the GST-catalyzed reaction between reduced glutathione (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, except theta). Under certain conditions, the interaction between glutathione and CDNB is totally dependent on the presence of active GST.

The GST-catalyzed formation of GS-DNB produces a dinitrophenyl thioether which can be detected by spectrophotometry at OD 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 purified protein fractions, and can quantitate GST-tagged fusion proteins. The detection limit of this product is < 1mU active GST.

Glutathione S-transferase (GST) is a family of enzymes that plays an important role in detoxification of xenobiotics. GST catalyzes attachment of the thiol of glutathione to electrophiles. Glutathione is used to scavenge potentially toxic compounds including those produced as a result of oxidative stress and is part of the defense mechanism neutralizing the mutagenic, carcinogenic and toxic effects of such compounds.

2. ASSAY SUMMARY



**For kinetic mode detection, incubation time given in this summary is for guidance only.*

3. PRECAUTIONS

Please read these instructions carefully prior to beginning the assay.

- All kit components have been formulated and quality control tested to function successfully as a kit.
- We understand that, occasionally, experimental protocols might need to be modified to meet unique experimental circumstances. However, we cannot guarantee the performance of the product outside the conditions detailed in this protocol booklet.
- Reagents should be treated as possible mutagens and should be handle with care and disposed of properly. Please review the Safety Datasheet (SDS) provided with the product for information on the specific components.
- Observe good laboratory practices. Gloves, lab coat, and protective eyewear should always be worn. Never pipet by mouth. Do not eat, drink or smoke in the laboratory areas.
- All biological materials should be treated as potentially hazardous and handled as such. They should be disposed of in accordance with established safety procedures.

4. STORAGE AND STABILITY

Store kit at -20ºC in the dark immediately upon receipt. Kit has a storage time of 1 year from receipt, providing components have not been reconstituted.

Refer to list of materials supplied for storage conditions of individual components. Observe the storage conditions for individual prepared components in the Materials Supplied section.

Aliquot components in working volumes before storing at the recommended temperature. **Reconstituted components are stable for 2 months.**

5. LIMITATIONS

- Assay kit intended for research use only. Not for use in diagnostic procedures.
- Do not mix or substitute reagents or materials from other kit lots or vendors. Kits are QC tested as a set of components and performance cannot be guaranteed if utilized separately or substituted.

6. MATERIALS SUPPLIED

Item	Amount	Storage Condition (Before Preparation)	Storage Condition (After Preparation)
GST Assay Buffer	25 mL	-20°C	-20°C
GST Substrate (CDNB)	100 µL	-20°C	-20°C
Reducing Agent I/Glutathione (GSH, lyophilized)	2 x 17 mg	-20°C	-20°C
GST Enzyme Standard	10 µL	-20°C	-20°C

7. MATERIALS REQUIRED, NOT SUPPLIED

These materials are not included in the kit, but will be required to successfully perform this assay:

- Microplate reader capable of measuring absorbance at OD 340 nm
- MilliQ water or other type of double distilled water (ddH_2O)
- Pipettes and pipette tips, including multi-channel pipette
- Assorted glassware for the preparation of reagents and buffer solutions
- Vortex
- Heat bath or water bath
- Tubes for the preparation of reagents and buffer solutions
- 96 well plate with clear flat bottom
- Dounce homogenizer (if using tissue)
- Sonicator

8. TECHNICAL HINTS

- **This kit is sold based on number of tests. A 'test' simply refers to a single assay well. The number of wells that contain sample, control or standard will vary by product. Review the protocol completely to confirm this kit meets your requirements. Please contact our Technical Support staff with any questions.**
- Selected components in this kit are supplied in surplus amount to account for additional dilutions, evaporation, or instrumentation settings where higher volumes are required. They should be disposed of in accordance with established safety procedures.
- Avoid foaming or bubbles when mixing or reconstituting components.
- Avoid cross contamination of samples or reagents by changing tips between sample, standard and reagent additions.
- Ensure plates are properly sealed or covered during incubation steps.
- Ensure all reagents and solutions are at the appropriate temperature before starting the assay.
- Samples which generate values that are greater than the most concentrated standard should be further diluted in the appropriate sample dilution buffer.
- Make sure you have the right type of plate for your detection method of choice.
- Make sure all necessary equipment is switched on and set at the appropriate temperature.

9. REAGENT PREPARATION

- Briefly centrifuge small vials at low speed prior to opening

9.1. **GST Assay Buffer:**

Ready to use as supplied. Equilibrate to room temperature before use. Store at 4°C or -20°C.

9.2. **GST Substrate (CDNB) – in DMSO:**

Ready to use as supplied. Warm by placing in a 37°C bath for 1 – 5 minutes to thaw the DMSO solution before use. ***NOTE: DMSO tends to be solid when stored at -20°C, even when left at room temperature, so it needs to melt for few minutes at 37°C.*** Aliquot probe so that you have enough volume to perform the desired number of assays. Store at -20°C protected from light and moisture. Once the substrate is thawed, use with two months. Keep on ice while in use.

9.3. **Reducing Agent I/Glutathione (GSH):**

Reconstitute in 1 vial with 275 µL GST Assay Buffer – each vial is sufficient for 50 assays. Aliquot Reducing Agent I/glutathione so that you have enough volume to perform the desired number of assays. Remaining solution can be stored at -20°C for 1 week.

9.4. **GST Enzyme Standard:**

Ready to use as supplied. Aliquot standard so that you have enough volume to perform the desired number of assays. Store at -20°C.

Prior to use, dilute 1:100 by adding 2 µL positive control to 198 µL GST Assay Buffer. Keep on ice while in use.

10. SAMPLE PREPARATION

General Sample Information

- We recommend that you use fresh samples. If you cannot perform the assay at the same time, we suggest that you complete the Sample Preparation step before storing the samples. Alternatively, if that is not possible, we suggest that you snap freeze samples in liquid nitrogen upon extraction and store the samples immediately at -80°C. When you are ready to test your samples, thaw them on ice. Be aware however that this might affect the stability of your samples and the readings can be lower than expected.

10.1. **Cell (adherent or suspension) samples:**

10.1.1. Harvest the amount of cells necessary for each assay (initial recommendation = 2 x 10^6 cells).

10.1.2. Wash cells with cold PBS.

10.1.3. Resuspend cells in 100 µL of GST Assay Buffer.

10.1.4. Homogenize cells quickly by pipetting up and down a few times.

10.1.5. Centrifuge sample at 4°C at 10,000 x *g* for 15 minutes using a cold microcentrifuge to remove any insoluble material.

10.1.6. Collect supernatant and transfer to a new tube.

10.1.7. Keep on ice.

10.2. **Tissue Samples:**

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

10.2.2. Harvest the amount of tissue necessary for each assay (initial recommendation = 100 mg).

10.2.3. Wash tissue in cold PBS.

10.2.4. Resuspend tissue in 500 µL of GST Assay Buffer.

10.2.5. Homogenize tissue with a Dounce homogenizer sitting on ice, with 10 – 15 passes.

10.2.6. Centrifuge samples at 4°C at 10,000 x *g* for 15 minutes using a cold microcentrifuge to remove any insoluble material.

10.2.7. Collect supernatant and transfer to a new tube.

10.2.8. Keep on ice.

10.3. **Plasma Erythrocyte Sample Preparation:**

10.3.1. Centrifuge anticoagulant treated blood at 1,000 x *g* for 10 minutes at 4°C.

10.3.2. Transfer the top plasma layer (without disturbing the white buffy layer) to a new tube.

10.3.3. Keep on ice for immediate use in assay.

The remaining sample should be stored at -80°C for future use and it is stable for 1 month.

10.4. **Plasma Erythrocyte Sample Preparation:**

10.4.1. Centrifuge anticoagulant treated blood at 1,000 x *g* for 10 minutes at 4°C.

10.4.2. Remove the white buffy later and discard – this layer contains leukocytes.

10.4.3. Lyse the erythrocytes (red blood cells) in 4 times volume of ice-cold GST Assay Buffer.

10.4.4. Centrifuge at 10,000 x g for 15 minutes at 4°C.

10.4.5. Collect supernatant (erythrocyte lysate) and transfer to a new tube.

10.4.6. Keep on ice.

The remaining samples should be stored at -80°C for future use and is stable for at least 1 month.

10.5. **Preparation of Bacterially Expressed GST-Fusion Protein Sample:**

10.5.1. Collect bacteria by centrifugation. Freeze/thaw the pellet twice, then sonicate in GST Assay Buffer.

10.5.2. Centrifuge at 10,000 x g for 15 minutes at 4°C.

10.5.3. Collect supernatant and transfer to a new tube.

10.5.4. Keep on ice.

The remaining samples should be stored at -80°C for future use and is stable for at least 1 month.

11. ASSAY PROCEDURE

- **Equilibrate all materials and prepared reagents to correct temperature prior to use.**
- **We recommended to assay all controls and samples in duplicate.**
- **Prepare all reagents and samples as directed in the previous sections.**

11.1. **Set up Reaction Wells**

Sample wells = 5 – 50 µL samples (adjust volume to 50 µL/well with GST Assay Buffer).

Positive control wells= 2 - 10 µL Diluted Positive control (adjust volume to 50 µL/well with GST Assay Buffer).

Background control wells = 50 µL GST Assay Buffer

11.2. Add 5 µL of Reducing Agent I/Glutathione to each sample and control well.

11.3. **GST Reaction Mix:**

11.3.1. Prepare 50 µL of Reaction Mix for each reaction. Mix enough reagents for the number of assays (samples and controls) to be performed. Prepare a master mix of the Reaction Mix to ensure consistency. We recommend the following calculation: X µL component x (Number reactions +1).

Components	Reaction Mix (µL)
GST Assay Buffer	49
GST Substrate (CDNB) Solution	1

11.3.2. Add 50 µL of Reaction Mix into each sample and control sample wells. Mix well.

11.3.3. Mix well.

11.4. Plate measurement:

11.4.1. Shake the plate carefully to start the reaction.

11.4.2. Measure output at OD340 nm on a microplate reader in a kinetic mode, every 2 – 3 minutes, for at least 10 minutes at RT protected from light, to obtain at least 5 time points.

Increase incubation reaction time for samples with low GST activity.

NOTE: *Incubation time depends on the GST Activity in the samples. We recommend measuring OD in a kinetic mode, and choosing two time points (T1 and T2) in the linear range (OD values A1 and A2 respectively) to calculate the GST activity of the samples.*

12. CALCULATIONS

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

12.2. Calculate the change in absorbance (ΔA340) per minute by selecting 2 points on the linear portion of the curve and determine the change in absorbance during the time using the following equation:

$$\Delta A340/\text{min} = \frac{A340\ (Time\ 2) - A340\ (Time\ 1)}{Time\ 2\ (min) - Time\ 1\ (min)}$$

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

12.4. Average the duplicate reading for each sample.

12.5. 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).

$$GST\ Activity := \left(\frac{\Delta A_{340}\ min^{-1} x\ Reaction\ Volume\ (mL)}{0.0096\ \mu mol^{-1} cm^{-1}\ x\ 1000\ mL\ x\ 0.2893\ cm\ x\ V}\right) * D$$

$$= \Delta A340 \text{min}^{-1} \text{ x } 0.036\text{x D/V } (\mu\text{mol/min/ml}$$

Where:

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

D = Sample dilution factor.

V = Sample Volume added to well (mL).

NOTE: 0.2893 cm is light path of the 0.1 mL Reaction Volume in a Greiner Bio One 655101 96-well plate (cm). If using other plates, they 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

13. TYPICAL DATA

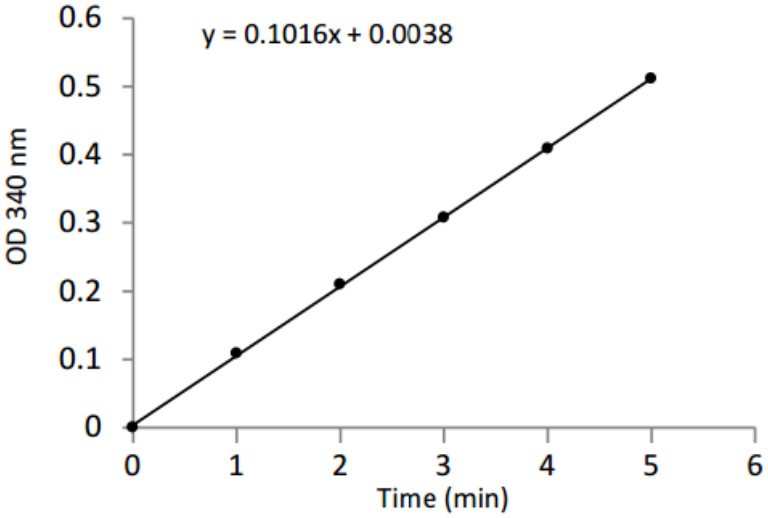


Figure 1: Typical GST kinetic assay curve using colorimetric reading.

Activity = (0.036 x 0.1016 x 100)/0.002 = 183 U/mL

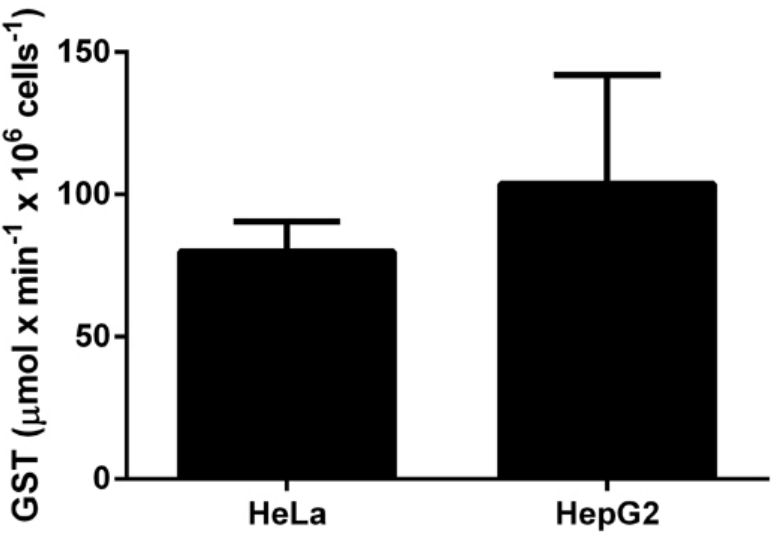


Figure 2: GST activity calculated in cell lysates, with background signal subtracted (duplicates +/- SD), using equation in protocol.

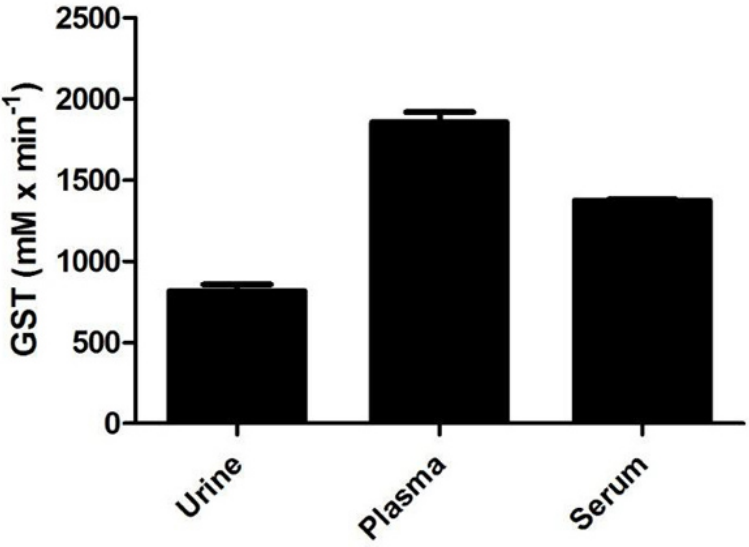


Figure 3: GST activity levels in human saliva, mouse serum and mouse plasma samples. Interpolated values from two concentrations were multiplied by their dilution factor to represent the level in a neat sample. Background subtracted data is plotted.

14. QUICK ASSAY PROCEDURE

NOTE: This procedure is provided as a quick reference for experienced users. Follow the detailed procedure when performing the assay for the first time.

- Prepare substrate, Reducing Agent I/glutathione and positive control (aliquot if necessary); get equipment ready.
- Prepare samples in duplicate.
- Set up plate for samples (50 µL) and positive control (50 µL) and background wells (50 µL)..
- Add 5 µL of Reducing Agent I/Glutathione to each well.
- Prepare XXXX Reaction Mix (Number samples + positive controls + 1).

Component	Reaction Mix (µL)
GST Assay Buffer	49
GST Substrate (CDNB) Solution	1

- Add 50 µL of GST Reaction Mix to control and sample wells.
- Measure plate at OD 340 nm at RT for 10 - 30 minutes.

15. 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

Problem	Cause	Solution
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

16. INTERFERENCES

These chemicals or biological materials will cause interference in this assay causing compromised results or complete failure:

- RIPA buffer – it contains SDS which can destroy/decrease the activity of the enzyme.

17. NOTES

Technical Support

For all technical or commercial enquiries please go to:
www.abcam.com/contactus
www.abcam.cn/contactus (China)
www.abcam.co.jp/contactus (Japan)