Gentaur
Gentaur Europe BVBA Voortstraat 49, 1910 Kampenhout BELGIUM
Tel 0032 16 58 90 45 info@gentaur.com

BioVision

For research use only

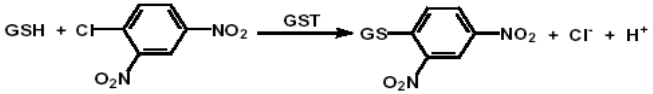
rev.1/16

GST Colorimetric Activity Assay Kit

(Catalog #K263-100; 100 assays; Store kit at -20°C)

I. Introduction:

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. The *GST Colorimetric Activity Assay Kit* 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, 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 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. Detect limit: Active GST < 1mU.

II. Kit Contents:

Component	K263-100	Cap Code	Part Number
GST Assay Buffer	25 ml	WM	K263-100-1
GST Substrate (CDNB)	0.1 ml	Red	K263-100-2
Glutathione (GSH, lyophilized)	2 x 17 mg	Yellow	K263-100-3
GST Positive Control	10 µl	Green	K263-100-4

III. Reagent Preparation and Storage Conditions:

GST Assay Buffer: store 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

IV. Sample Preparation Guideline:

A. Cell Sample Preparation:

1. Collect cells by centrifugation. For adherent cells, use a rubber policeman to scrape and collect the cells.
2. Homogenize or sonicate the cells in GST Assay Buffer (typically 3 -4 volumes).
3. Centrifuge at 10,000 x g for 15 min at 4°C.
4. 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. 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. Homogenize tissue in GST Assay Buffer (100 mg/0.5 ml).
3. Centrifuge at 10,000 x g for 15 minutes at 4°C.
4. 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. 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 its volume of ice-cold GST Assay Buffer.
5. Centrifuge at 10,000 x g for 15 min at 4°C.
6. 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. 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 10,000 x g for 15 min at 4°C.
3. 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.

V. GST Assay Protocol:

1. **Sample, Negative Control and Positive Control Preparation**: Prepare samples in a total 50 µl volume with GST Assay Buffer, including a negative control with 50 µl of GST Assay buffer only. For GST Positive Control, dilute 100 time by adding 2 µl of Positive Control into 198 µl GST Assay Buffer, add 2-10 µl of diluted GST Positive Control into desired well (s) and adjust the final volume to 50 µl with GST Assay Buffer.
   Note: 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 reagents for the number of assays to be performed. For each well, prepare a total 50 µl Substrate Mix containing:

   GST Assay Buffer 49 µl
   GST Substrate (CDNB) Solution 1 µl

   Mix well and transfer 50 µl of the Mix into each sample wells (Negative, Positive controls and samples).
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

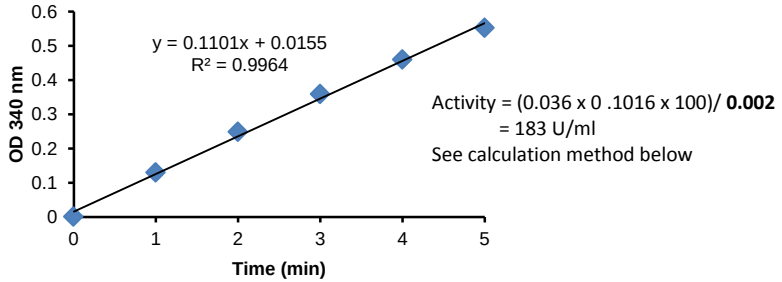


Figure: GST Kinetic Assay Performed According to This Protocol

5. **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:

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

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).

$$\text{GST Activity} = \frac{\Delta A_{340}min^{-1} \times \text{Reaction Volume (ml)}}{0.0096\ \mu mol^{-1}cm^{-1} \times 1000\ ml \times 0.2893\ cm \times V} \times D$$

$$= \Delta A_{340}min^{-1} \times 0.036 \times D/V\ (\mu mol/min/ml$$

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.

RELATED PRODUCTS:

Apoptosis Detection Kits & Reagents
- Annexin V Kits & Bulk Reagents
- Caspase Assay Kits & Reagents
- Mitochondrial Apoptosis Kits & Reagents
- Nuclear Apoptosis Kits & Reagents

Cell Fractionation System
- Mitochondria/Cytosol Fractionation Kit
- Nuclear/Cytosol Fractionation Kit
- Membrane Protein Extraction Kit
- Cytosol/Particulate Rapid Separation Kit
- Mammalian Cell Extraction Kit
- FractionPREP Fractionation System

Cell Proliferation & Senescence
- Quick Cell Proliferation Assay Kit
- Senescence Detection Kit
- High Throughput Apoptosis/Cell Viability Assay Kits
- LDH-Cytotoxicity Assay Kit
- Bioluminescence Cytotoxicity Assay Kit
- Live/Dead Cell Staining Kit

Cell Damage & Repair
- HDAC Fluorometric & Colorimetric Assays & Drug Discovery Kits
- HAT Colorimetric Assay Kit & Reagents
- DNA Damage Quantification Kit
- Glutathione & Nitric Oxide Fluorometric & Colorimetric Assay Kits

Signal Transduction
- cAMP & cGMP Assay Kits
- Akt & JNK Activity Assay Kits
- Beta-Secretase Activity Assay Kit

Adipocyte & Lipid Transfer
- Recombinant Adiponectin, Survivin, & Leptin
- CETP Activity Assay & Drug Discovery Kits
- Total Cholesterol Quantification Kit

Molecular Biology & Reporter Assays
- siRNA Vectors
- Cloning Insert Quick Screening Kit
- Mitochondrial & Genomic DNA Isolation Kits
- 5 Minutes DNA Ligation Kit
- 20 Minutes Gel Staining/Destaining Kit

Growth Factors and Cytokines
Monoclonal and Polyclonal Antibodies and Cytokines

FOR RESEARCH USE ONLY! Not to be used in humans.

GENERAL TROUBLESHOOTING GUIDE:

Problems	Cause	Solution
Assay not working	• Use of ice-cold assay buffer	• Assay buffer must be at room temperature
	• Omission of a step in the protocol	• Refer and follow the data sheet precisely
	• Plate read at incorrect wavelength	• Check the wavelength in the data sheet and the filter settings of the instrument
	• Use of a different 96-well plate	• Fluorescence: Black plates (clear bottoms) ; Luminescence: White plates ; Colorimeters: Clear plates
Samples with erratic readings	• Use of an incompatible sample type	• Refer data sheet for details about incompatible samples
	• Samples prepared in a different buffer	• Use the assay buffer provided in the kit or refer data sheet for instructions
	• Cell/ tissue samples were not completely homogenized	• Use Dounce homogenizer (increase the number of strokes); observe for lysis under microscope
	• Samples used after multiple free-thaw cycles	• Aliquot and freeze samples if needed to use multiple times
	• Presence of interfering substance in the sample	• Troubleshoot if needed
	• Use of old or inappropriately stored samples	• Use fresh samples or store at correct temperatures until use
Lower/ Higher readings in Samples and Standards	• Improperly thawed components	• Thaw all components completely and mix gently before use
	• Use of expired kit or improperly stored reagents	• Always check the expiry date and store the components appropriately
	• Allowing the reagents to sit for extended times on ice	• Always thaw and prepare fresh reaction mix before use
	• Incorrect incubation times or temperatures	• Refer datasheet & verify correct incubation times and temperatures
	• Incorrect volumes used	• Use calibrated pipettes and aliquot correctly
Readings do not follow a linear pattern for Standard curve	• Use of partially thawed components	• Thaw and resuspend all components before preparing the reaction mix
	• Pipetting errors in the standard	• Avoid pipetting small volumes
	• Pipetting errors in the reaction mix	• Prepare a master reaction mix whenever possible
	• Air bubbles formed in well	• Pipette gently against the wall of the tubes
	• Standard stock is at an incorrect concentration	• Always refer the dilutions in the data sheet
	• Calculation errors	• Recheck calculations after referring the data sheet
	• Substituting reagents from older kits/ lots	• Use fresh components from the same kit
Unanticipated results	• Measured at incorrect wavelength	• Check the equipment and the filter setting
	• Samples contain interfering substances	• Troubleshoot if it interferes with the kit
	• Use of incompatible sample type	• Refer data sheet to check if sample is compatible with the kit or optimization is needed
	• Sample readings above/below the linear range	• Concentrate/ Dilute sample so as to be in the linear range
Note: The most probable list of causes is under each problem section. Causes/ Solutions may overlap with other problems.		