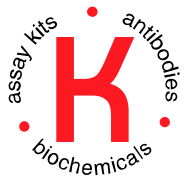

KAMIYA BIOMEDICAL COMPANY

GST Colorimetric Activity Assay

For colorimetric determination of GST activity in plasma, cell and tissue homogenates

Cat. No. KT-204

For Research Use Only. Not for Use in Diagnostic Procedures.

PRODUCT INFORMATION

GST Colorimetric Activity Assay
Cat. No. KT-204

PRODUCT
The **K-*ASSAY*®** GST Colorimetric Activity Assay is for colorimetric determination of GST activity in plasma, cell and tissue homogenates.

PRINCIPLE
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. The **K-*ASSAY*®** GST Colorimetric Activity Assay is based upon the GST-catalyzed reaction between GSH and 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.

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 purified protein fraction and also quantitate GST-tagged fusion protein. Detection limit: Active GST < 4 mU.

COMPONENT
- GST Assay Buffer 10 mL
- GST Sample Buffer 25 mL
- GST Substrate (CDNB) 1 mL
- Glutathione (GSH, lyophilized) 2 x 17 mg
- GST Positive Control (0.625 µg/µL) 20 µL

PROTOCOLS
Reagent Preparation and Storage
GSH: Add 550 µL of GST Sample Buffer to each vial just before use. One vial is sufficient for 50 assays. Remaining solution can be kept at –20°C for 1 week.

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

Sample Preparation Guideline
A. Cell Sample Preparation
1. Collect cells by centrifugation. For adherent cells, use a rubber policeman scraping the cells to collect.
2. Homogenize or sonicate the cells in GST Sample Buffer.
3. Centrifuge at 10,000 X g for 15 minutes at 4°C.
4. Collect supernatant and use for the assay. The remaining sample can be stored at –80°C, 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 Sample 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, stable for at least 1 month.

C. Plasma and Erythrocyte Sample Preparation

1. Centrifuge an anticoagulant treated blood at 1,000 x g for 10 minutes at 4°C.
2. Transfer the top plasma layer (without disturbing the white puffy 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 Sample Buffer.
5. Centrifuge at 10,000 x g for 15 minutes 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, stable for at least 1 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 Sample Buffer.
2. Centrifuge at 10,000 x g for 15 minutes 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, stable for at least 1 month.

ASSAY PROTOCOL

1. Prepare sample in a total 100 µL volume with GST Sample Buffer, including a negative control with 100 µL of GST Sample Buffer only and a positive control with 2 µL of GST and 98 µL of Sample Buffer.
   Note: We recommend preparing several dilutions of your sample and perform duplicate wells for each measurement.
2. Add 10 µL of Glutathione to each well containing the sample or control above.
3. Prepare Substrate Mix by adding 10 µL CDNB Solution + 90 µL of GST Assay Buffer for each sample including the positive control.
4. Mix well and transfer 100 µL for the mix into each sample (including the positive control) well.
5. Carefully shake the plate to start the reaction.
6. Read the absorbance once every minute at 340 nm using a plate reader to obtain at least 5 time points.

CALCULATION OF RESULTS

1. Determine the change in absorbance (ΔA340) 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. Select two points on the linear portion of the curve and determine the change in absorbance during that time, using the following equation:

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

2. Determine the rate of ΔA340/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. 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 (0.524 cm).

$$\text{GST Activity} = \frac{\Delta A_{340}\ min^{-1} \times 0.2\ mL\ Reaction\ Volume}{0.0096\ \mu mol^{-1}\ cm^{-1} \times 1000\ mL \times 0.524\ cm \times A} \times \text{Sample Dilution}$$

$$= 0.0396 \times \Delta A_{340}\ min^{-1} \times \text{Sample Dilution}/A \quad (\mu mol/min)$$

Where:

0.0096 μM^{-1} cm^{-1} is GS-DNB extinction coefficient.
A is sample volume in the reaction well in milliliter (mL).
0.524 cm is light path of the 0.2 mL Reaction Volume in 96 well plate (cm).

STORAGE

Store at -20°C. The kit is stable until the expiration date shown on the label when stored at -20°C.

FOR RESEARCH USE ONLY

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