QuantiChrom™ Glutathione S-transferase Assay Kit (DGST-100)

Quantitative Colorimetric Glutathione S-transferase Determination

DESCRIPTION

GLUTATHIONE TRANSFERASE (GST; EC 2.5.1.18) is a multifunctional enzyme that plays an important role in cellular detoxification. GST protects cells against foreign compounds such as carcinogens and drugs by catalyzing the attachment of glutathione to the compounds electrophilic and/or hydrophobic sites.

BioAssay Systems' Glutathione S-transferase assay kit is based on the GST enzyme reaction between GSH and the GST substrate, CDNB (1-chloro-2,4-dinitrobenzene). The GST catalyzed formation of GS-DNB produces a dinitrophenyl thioether which can be detected spectrophotometrically at 340 nm. The rate of increase in absorbance at 340 nm is directly proportional to the GST activity in the sample.

KEY FEATURES

Fast and sensitive. Linear detection range (20 µL sample): 2 to 80 U/L for 10 min reaction at 25˚C.

Convenient and high-throughput. Homogeneous "mix-incubate-measure" type assay. Can be readily automated on HTS liquid handling systems for processing thousands of samples per day.

APPLICATIONS

Glutathione S-transferase activity determination in biological samples (e.g. cell lysates, tissues, etc.)

KIT CONTENTS (100 TESTS IN 96-WELL PLATES)

Assay Buffer: 25 mL **Glutathione:** Powder
CDNB: 120 µL

Storage conditions. The kit is shipped at room temperature. Store all components at 4°C upon receiving. Shelf life: 6 months after receipt.

Precautions: Reagents are for research use only. Normal precautions for laboratory reagents should be exercised while using the reagents. Please refer to the Material Safety Data Sheet for detailed information.

PROCEDURES

This assay is based on a kinetic reaction. To ensure identical incubation time, addition of Working Reagent to samples should be quick and mixing should be brief but thorough. Use of a multi-channel pipettor is recommended. Assays can be executed at any desired temperature (e.g. 25°C or 37°C).

Sample Preparation:

Tissue: prior to dissection, rinse tissue in phosphate buffered saline (pH 7.4) to remove blood. Homogenize tissue (50 mg) with a Dounce homogenizer in ~250 µL cold 100 mM potassium phosphate, pH 7.0 containing 2 mM EDTA. Freeze the homogenized tissue at –80°C to lyse the cells. After freezing, thaw and centrifuge samples at 10,000×*g* for 15 minutes at 4°C. Remove supernatant for assay.

Cell Lysate: collect cells (~4 millions cells) by centrifugation at 2,000×*g* for 5 min at 4°C. For adherent cells, do not harvest cells using proteolytic enzymes; rather use a rubber policeman. Homogenize or sonicate cells in an appropriate volume of cold buffer containing 100 mM potassium phosphate (pH 7.0) and 2 mM EDTA. Centrifuge at 10,000×*g* for 15 min at 4°C. Remove supernatant for assay.

All samples can be stored at –20 to –80°C for at least one month.

Assay Procedure:

1. *Reagent Preparation.* Bring all reagents to the desired reaction temperature (e.g. 25˚C) prior to assay. Briefly centrifuge tubes before use.

   Reconstitute Glutathione tube with 120 µL dH_2O. Vortex tube to mix. Unused Glutathione reagent is stable for three weeks when stored frozen at -20°C.
2. Transfer 20 µL of each sample into separate wells.
3. Prepare enough Working Reagent (WR) for all reaction wells by mixing, for each 96-well assay, 184 µL Assay Buffer, 1 µL Reconstituted Glutathione, and 1 µL CDNB.

   Add 180 µL WR to all samples and tap plate briefly to mix.
4. Read OD_{340nm} at time 0 min and at least four other time points between 0 min and 10 min. If available we recommend reading the plate in a plate reader capable of kinetic measurements and set it to read the OD_{340nm} every min for 10 min.

CALCULATION

Plot the OD_{340nm} versus time and use OD values in the linear part to determine the GST activity in a sample which is computed as follows:

$$\text{GST Activity} = \frac{OD_{t2} - OD_{t1}}{t} \times \frac{1}{0.0096\ \mu M^{-1}cm^{-1} \cdot l} \times \frac{V_{total}}{V_{sample}} \times n$$

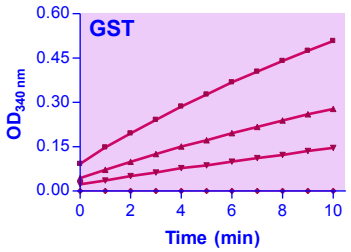
$$= \frac{OD_{t2} - OD_{t1}}{t} \times \frac{10}{0.00503\ \mu M^{-1}} \times n \quad (U/L)$$

where OD_{t2} and OD_{t1} are OD's at two different time points in the linear range of the curve and t is the time difference between the two time points. For example, if measurements at t = 0 and t = 10 min are used, then in the equation OD_{t1} is the OD at 0 min, OD_{t2} is the OD at 10 min. and t = 10. The extinction coefficient of GS-DNB is 0.0096 $\mu M^{-1}cm^{-1}$ which becomes 0.00503 μM^{-1} when multiplied by the path-length for 200 µL in a 96 well plate (0.524 cm). Total Reaction Volume (V_{total}) = 200 µL and Sample Volume (V_{sample}) = 20 µL. n is the sample dilution factor. It is prudent to test several dilutions to determine an optimal dilution factor n.

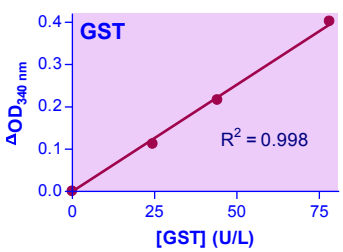
Unit definition: one unit of enzyme will conjugate 1 µmole of CDNB per min under the assay conditions.

MATERIALS REQUIRED, BUT NOT PROVIDED

Pipetting devices, centrifuge tubes, clear flat-bottom 96-well plates (e.g. VWR cat# 82050-760), and plate reader.



Raw Kinetic Data



GST Titration

LITERATURE

1. Csiszár, Jolán, et al. (2014). Glutathione transferase supergene family in tomato: Salt stress-regulated expression of representative genes from distinct GST classes in plants primed with salicylic acid. *Plant Physiology and Biochemistry* 78: 15-26.
2. Smeyne, Michelle, and Richard Jay Smeyne. (2013). Glutathione metabolism and Parkinson's disease. *Free Radical Biology and Medicine* 62: 13-25.
3. Watson, Mary A., et al. (1998). Human glutathione S-transferase P1 polymorphisms: relationship to lung tissue enzyme activity and population frequency distribution. *Carcinogenesis* 19.2: 275-280.