Glutathione S-transferase Activity Assay Kit (Colorimetric)

Glutathione S-transferase Activity Assay Kit (Colorimetric) can be used to measure Glutathione S-transferase activity in cell lysates and tissues.

Catalog number: ARG82167

Package: 100 tests

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INTRODUCTION

Glutathione S-transferase (GST), previously known as ligandins, are a family of eukaryotic and prokaryotic phase II metabolic isozymes best known for their ability to catalyze the conjugation of the reduced form of glutathione (GSH) to xenobiotic substrates for the purpose of detoxification. The GST family consists of three superfamilies: the cytosolic, mitochondrial, and microsomal—also known as MAPEG—proteins. Members of the GST superfamily are extremely diverse in amino acid sequence, and a large fraction of the sequences deposited in public databases are of unknown function. The Enzyme Function Initiative (EFI) is using GSTs as a model superfamily to identify new GST functions. [Provide by Wikipedia: Glutathione S-transferase]

PRINCIPLE OF THE ASSAY

This Glutathione S-transferase Activity Assay Kit (Colorimetric) is a simple colorimetric assay that measures the amount of Glutathione S-transferase (GST) present in biological samples. This assay 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 O.D. 340 nm. The rate of increase in absorbance at O.D. 340 nm is directly proportional to the GST activity in the sample.

MATERIALS PROVIDED & STORAGE INFORMATION

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

Component	Quantity	Storage information
Assay Buffer (pH 7.0)	25 mL	4°C
CDNB	120 µL	4°C
Glutathione (lyophilized)	1 vial	4°C

MATERIALS REQUIRED BUT NOT PROVIDED

- Microplate reader capable of reading at O.D. 405 nm
- Clear flat-bottom 96 well microplate
- Deionized or Distilled water
- Pipettes, pipette tips and Multichannel micropipette reservoir

TECHNICAL NOTES AND PRECAUTIONS

- Wear protective gloves, clothing, eye, and face protection especially while handling blood or body fluid samples.
- 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).
- Reagents are for research use only. Normal precautions for laboratory reagents should be exercised while using the reagents. Please refer to Material Safety Data Sheet for detailed information.
- All reagents should be mixed by gentle inversion or swirling prior to use. Do not induce foaming.
- Before using the kit, spin tubes and bring down all components to the bottom of tubes.
- It is highly recommended assaying the Standards and samples in duplicates.
- Change pipette tips between the addition of different reagent or samples.

SAMPLE COLLECTION & STORAGE INFORMATION

The sample collection and storage conditions listed below are intended as general guidelines. Sample stability has not been evaluated.

Tissue lysate: rinse tissue in phosphate buffered saline (pH 7.4) to remove blood. Homogenize tissue (50 mg) with a Dounce homogenizer in ~250 µL of 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 x g for 15 minutes at 4°C. Collect supernatant for assay.

Cell lysate: collect cells (~4 millions cells) by centrifugation at 2,000 x g for 5 min at 4°C. 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 x g for 15 minutes at 4°C. Collect supernatant for assay.

Note:

- For adherent cells, do not harvest cells using proteolytic enzymes; rather use a rubber policeman.
- All samples can be stored at -20 to -80°C for at least one month.

REAGENT PREPARATION

- **Reconstitute Glutathione:** add 120 µL of distilled water into the Glutathione tube. Vortex tube to mix. Unused Glutathione reagent is stable for three weeks when stored frozen at-20°C.
- **Working Reagent:** for each well, mixing 1 µL of CDNB, 1 µL of Reconstitute Glutathione and 184 µL of Assay Buffer. Fresh reconstitution is recommended.

ASSAY PROCEDURE

Equilibrate reagents to room temperature or 37°C. Briefly centrifuge tubes before use.

	Sample well
Samples	20 µL
Working Reagent	180 µL
Read the absorbance at **O.D. 340 nm** for **time 0 minute** and at least four **other time points between 0 minute and 10 minutes.** If available we recommend reading the plate in a plate reader capable of kinetic measurements and set it to read the O.D. 340 nm every minute for 10 minutes.	

CALCULATION OF RESULTS

1. Plot the O.D. 340 nm versus time and use O.D. values in the linear part to determine the GST activity in a sample which is calculated as follows:

   GST Activity (U/L)

   $= [(OD_{t2} - OD_{t1}) / t] \times [1 / (0.0096\ \mu M^{-1}cm^{-1} \times l)] \times (V_{Total} / V_{Sample}) \times n$

   $= [(OD_{t2} - OD_{t1}) / t] \times (10 / 0.00503\ \mu M^{-1}) \times n$

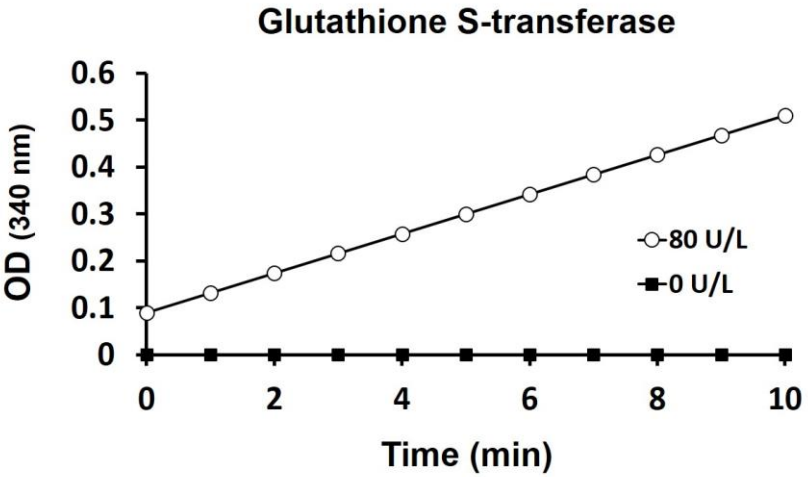
   Note:

   - OD_{t2}, OD_{t1}: the O.D. 340 nm values at two different time points in the linear range of the curve and t is the time difference between the two time points. (E.g., if measurements at t = 0 and t = 10 minutes are used, then in the equation OD_{t1} is the OD at 0 minute, OD_{t2} is the OD at 10 minutes. 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.

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

EXAMPLE OF TYPICAL STANDARD CURVE

The following figures demonstrate typical results with the Glutathione S-transferase Activity Assay Kit (Colorimetric). One should use the data below for reference only. This data should not be used to interpret actual results.



QUALITY ASSURANCE

Sensitivity

2 U/L