BIOMEDICAL RESEARCH SERVICE CENTER
UNIVERSITY at BUFFALO, STATE UNIVERSITY of NEW YORK

Department of Biochemistry, Attn: Dr. Lee, University at Buffalo, 3435 Main Street, Buffalo, NY 14214, USA
Tel/Fax: (716) 829-3106 Email: chunglee@buffalo.edu Web: www.bmrservice.com

Glutathione S-Transferase (GST) Assay Kit (Cat #: E-127)

COMPONENTS: 10x Cell Lysis Solution: 25 ml, store at 4ºC **(swirl bottle briefly prior to pipetting)**
50x GSH Buffer: 1 ml, store at 4ºC
200x CDNB: 0.25 ml, store at -20°C (for 250 assays)
GSH: 0.35 g, store at 4ºC

PRODUCT DESCRIPTION: The GST assay kit is based on 1-chloro-2,4-dinitrobenzene (CDNB) conjugation to the thiol group of glutathione (PNAS 71:3879-3882, 1974), and can be employed to measure the total GST activity of a crude cell/tissue extract and plasma. Glutathione conjugation to CDNB causes increased absorbance at 340 nm (ε = 9.6 $mM^{-1}cm^{-1}$). The assay requires a UV-transparent 96-well plate for measuring kinetic changes in $O.D._{340\ nm}$.

Preparation of cell/tissue extracts:

1. Wash $10^5 - 10^6$ cells with ice-cold phosphate-buffered saline (PBS). Animal tissue should be washed with PBS thoroughly to remove blood cells. Freeze cell pellet and tissue at -80ºC until use.
2. Prepare enough 1x Cell Lysis Solution by diluting 10x Cell Lysis Solution with ice-cold dH_2O. Add 50 – 100 µl 1x Cell Lysis Solution to cell pellet. Extract cells by pipetting up and down (gently but thoroughly). Leave lysate on ice for 5 min with intermittent gentle agitation. Centrifuge lysate in a refrigerated microfuge for 3 min at maximum speed (~13,000 rpm) and harvest supernatant for Catalase assay. For tissue extraction, weigh ~25 mg tissue and homogenize in 0.5 ml ice-cold 1x Cell Lysis Solution. Centrifuge homogenate at 4ºC for 3 min at maximum speed and harvest supernatant. Store cell lysate and tissue homogenate at -80ºC.
3. Perform protein assay to determine sample protein concentration. Normalize sample protein concentration with ice-cold 1x Cell Lysis Solution. Note: do not use a buffer containing reducing agents or SDS.

Preparation of assay solutions:

0.2M GSH (glutathione) - Prepare GSH solution by dissolving 12 mg GSH in 0.2 ml ice-cold ddH_2O. GSH solution, if stored in aliquots at -80°C, is good for several months. Keep solution on ice during assay.

Substrate Solution- Calculate the volume of Substrate Solution required for each experiment. Each sample requires 0.2 ml Substrate Solution. Use the freshly prepared Substrate Solution IMMEDIATELY. Substrate Solution is prepared using the following mixing ratio: 1 ml dH_2O, 20 µl 50x GSH Buffer, 20 µl 0.2M GSH and finally 5 µl CDNB. Immediately vortex solution to prevent CDNB precipitation. Scale up the volume proportionately.
Note: Add CDNB to Substrate Solution after plate reader is set up in kinetic mode.

Assay:

1. Use a UV-transparent 96-well plate for kinetic measurement of $O.D._{340\ nm}$. Set up the plate reader in kinetic mode. Pipette 10 µl sample to each well. Proceed to prepare Substrate Solution and use it IMMEDIATELY. Reaction is initiated by addition of 0.2 ml Substrate Solution to each well. Gently agitate plate for ~10 sec and then begin recording $O.D._{340\ nm}$ every 30 sec for 5 min. The increase in O.D.340 nm is proportional to GST activity.
2. Generate a plot of assay time vs. $O.D._{340\ nm}$. Use the linear portion of the curve to obtain a trend line equation **(y = mx + b)**. The slope **m** represents sample GST activity. Alternatively, use the molar extinction coefficient ($\boldsymbol{\varepsilon}$ = 9.6 $mM^{-1}cm^{-1}$) and the slope **m** to calculate sample GST specific activity as described in step 3.
3. If the linear portion spans over the first 3 min and using a 0.6-cm light path for the 96-well plate, sample GST specific activity can be expressed as µmol/ml/min **= (m × 20) ÷ (9.6 $mM^{-1}cm^{-1}$ × 0.6 cm x 3 min) = 1.157 × m**

Additional information:

The equation may be adjusted if a different light path length or assay time is used for O.D. measurement. Please contact us or visit the product web page for MSDS information of CDNB and DMSO.