BIOMEDICAL RESEARCH SERVICE CENTER
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Glutathione S-Transferase (GST) Assay Kit (Cat #: E-127)

COMPONENTS: 50x GSH Buffer: 1 ml, store at 4ºC
200x CDNB: 0.25 ml, store at -20°C (for 250 assays; **contains DMSO**)
GSH: 0.35 g, store at 4ºC
10x Cell Lysis Solution- 25 ml, store at 4ºC **(contains 1% TX-100; swirl bottle briefly prior to dilution)**

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 total GST activity of cell/tissue extracts. 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 or a 0.2-ml quartz cuvette for measuring kinetic changes in $O.D._{340\ nm}$.

Preparation of cell/tissue extracts:

1. Prepare 1x Cell Lysis Solution by diluting 10x Cell Lysis Solution with ice-cold dH_2O. Bring up at least ~10^5 washed cells in 100 – 200 µl ice-cold 1x Cell Lysis Solution by pipetting up and down gently. Leave lysate on ice for 5 min with agitation. If lysate is overly turbid, add more 1x Cell Lysis Solution and repeat pipetting. Tissue is homogenized in ice-cold 1x Cell Lysis Solution (~10 mg tissue in 0.5 ml).
2. Centrifuge lysate in a cold microfuge at ~14,000 rpm for 5 min. Supernatant is harvested and stored at -80ºC.
3. Use the BCA protein assay method to determine lysate protein concentration. A suggested sample protein concentration range is 0.5 – 1 mg/ml.

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 freshly prepared Substrate Solution IMMEDIATELY. Substrate Solution is prepared using the following mixing ratio: 0.96 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: CDNB should be added to Substrate Solution after the plate reader or spectrophotometer is set up.

Assay Protocol:

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 (ε = 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 1-cm light path for the 96-well plate, sample GST specific activity can be expressed as µmol/ml/min $= (m \times 20) \div (9.6\ mM^{-1}cm^{-1} \times 1\ cm \times 3\ min)$
$= 0.69 \times m$

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.