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

COMPONENTS: 10x Cell Lysis Solution: 25 ml, store at 4℃
50x GSH/GST Buffer: 1 ml, store at room temperature
200x CDNB: 0.25 ml, store at -20℃ (for 250 assays)
GSH: 0.35 g, store at 4℃

PRODUCT DESCRIPTION: The glutathione S-transferase (GST) family comprises multiple classes of enzymes with diverse functions. GSTs are known for their ability to catalyze the conjugation of reduced glutathione (GSH) to electrophiles on many substrates and mediate GSH-dependent isomerization reactions. The GST assay kit is based on CDNB (PNAS 71:3879-3882, 1974), and can be employed to measure the activity of a purified GST enzyme or total GST activity of a crude cell/tissue extract and plasma. GSH 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 0.2-ml Quartz cuvette 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 -80℃ 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 ~50 mg tissue and homogenize in 1 ml ice-cold 1x Cell Lysis Solution. Centrifuge homogenate at 4℃ for 3 min at maximum speed and harvest supernatant. Store cell lysate and tissue homogenate at -80℃.
3. Perform protein assay to determine sample protein concentration. Dilute sample protein concentration with ice-cold 1x Cell Lysis Solution to 1 mg/ml.

Preparation of assay solutions:

GSH- Prepare a 0.2M GSH solution by dissolving 12 mg GSH in 0.2 ml ice-cold ddH_2O. The GSH solution, if stored in aliquots at -80℃, is good for several months. Keep GSH solution on ice during assay.

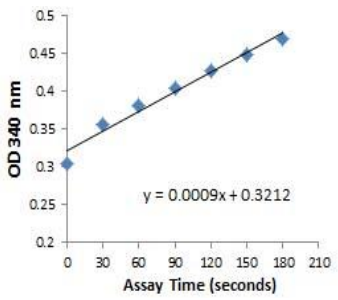
Working solution- Each assay requires 0.2 ml working solution. Calculate the amount of working solution required for each assay and use the freshly prepared solution as soon as possible (within 5 min). Working solution is prepared using the mixing ratio: 1 ml dH_2O, 20 µl 50x GSH/GST Buffer, 20 µl 0.2M GSH and 5 µl CDNB. Immediately vortex solution to prevent CDNB precipitation. Scale up the volume proportionately.

If using a Quartz cuvette for measurement, working solution will need to be prepared separately for each measurement using the mixing ratio: 0.2 ml dH_2O, 4 µl 50x GSH/GST Buffer, 4 µl 0.2M GSH and 1 µl CDNB. Immediately vortex solution to prevent CDNB precipitation.

Kinetic Assay:

1. Use a UV-transparent 96-well plate (or a 0.2-ml Quartz cuvette) for kinetic measurement of $O.D._{340\ nm}$. Add 10 µl sample first. The reaction is initiated by addition of 0.2 ml working solution followed by mixing. Wait ~30 sec and then begin to record $O.D._{340\ nm}$ every 30 sec (up to 3 min). The increase in O.D.340 nm is proportional to GST activity.
2. Generate a plot of assay time (**in sec**) vs. $O.D._{340\ nm}$ (see representative graph). Generate the trend line equation **(y = mx + b)**. Use the molar extinction coefficient **ε** (9.6 $mM^{-1}cm^{-1}$) and the slope **m** (from the equation) for calculation of sample GST activity as follows.
3. Sample GST activity (**µmol/ml/min**) in 96-well format (0.5-cm path length)
   = (m × 60 sec × 20) ÷ (9.6 $mM^{-1}cm^{-1}$ × 0.5 cm) = 250 × m

   Sample GST activity (**µmol/ml/min**) in cuvette format (1-cm path length)
   = (m × 60 sec × 20) ÷ (9.6 $mM^{-1}cm^{-1}$ × 1 cm) = 125 × m



Additional information:

- The equation may be adjusted if a different light path is used for O.D. measurement.
- Please contact us or visit the product web page for MSDS information.