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

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Glutathione (GSH) Assay Kit (Cat #: A-126)

COMPONENTS: 50x GSH Buffer: 1 ml, store at 4°C
Glutathione (GSH): 0.1 g, store at 4°C
200x CDNB: 0.1 ml, store at -80°C (100 assays)
50x GST: 0.4 ml, store at -80°C (100 assays)

PRODUCT DESCRIPTION: Glutathione (GSH) is a tripeptide, γ-L-glutamyl-L-cysteinyl-glycine, involved in diverse cellular function. The GSH assay kit is based on a spectrophotometric assay (PNAS 71:3879-3882,1974), which employs 1-chloro-2,4-dinitrobenzene (CDNB) as a substrate for GST-mediated GSH conjugation. The conjugation causes an increase in the absorbance at 340 nm. The assay requires UV-transparent 96-well plates for measuring increased O.D.340 nm. Assay reagents are stable for several years if stored and handled properly.

PROTOCOL

Sample preparation by TCA extraction:

Cell and tissue samples can be extracted by TCA. Please follow detailed protocol at http://www.bmrservice.com/SupplementTCA.html. Note that TCA is highly corrosive and Ether is highly flammable. Please contact us or visit the product webpage for MSDS information.

Glutathione (GSH) standard:

First prepare an 8 mM GSH (4.9 mg GSH dissolved in 2 ml dH_2O). Dilute the solution 10-fold with dH_2O to obtain 0.8 mM GSH. Perform serial dilutions to obtain 0.4 mM, 0.2 mM and 0.1 mM GSH standards. GSH standards should be prepared fresh for each assay.

Reagent thawing:

Keep thawed 200x CDNB at room temperature and thawed 50x GST on ice. It is important to minimize the time that the reagents are thawed. Freeze the reagents IMMEDIATELY after use.

Preparation of working solution:

Estimate the volume of working solution required for each assay: 0.2 ml required per reaction (e.g., if preparing working solution for 10 reactions, first add 40 µl 50x GSH Buffer to 2 ml dH_2O. Then add 10 µl 200x CDNB and immediately vortex solution vigorously (initial cloudy appearance is normal). Finally add 40 µl 50x GST followed by gentle vortexing. Keep working solution on ice and use as soon as possible.

ASSAY

1. Add 20 µl dH_2O (blank), 20 µl of each GSH standard and 20 µl of each sample to a UV-transparent 96-well plate.
2. Add 0.2 ml freshly prepared working solution to each well, and mix contents by gently agitating plate for 30 sec. Incubate plate at 37°C for 20 min.
3. Measure $O.D._{340\ nm}$ using a plate reader. The blank reading should be subtracted from all GSH standard and sample readings. Presence of GSH will cause increased $O.D._{340\ nm}$ reading in a concentration-dependent manner.
4. Generate a standard plot of GSH concentrations vs. $O.D._{340\ nm}$. Apply sample readings to the standard curve to obtain GSH concentration. A new standard plot must be generated for each assay.