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

COMPONENTS: GSH Assay Solution: 50 ml, store at 4°C
Glutathione (GSH): 100 mg, store at 4°C
200x CDNB: 0.1 ml, store at -80°C
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 a UV-transparent 96-well plate or 0.2-ml quartz cuvette for measuring changes in $O.D._{340\ nm}$. Assay reagents are stable for several years if stored and handled properly.

PROTOCOLS

Sample preparation by TCA extraction:

Cell/tissue samples are deproteinized by TCA and extracted by diethyl ether prior to assay. The extraction protocol is available at https://bmrservice.com/deproteination-by-tca. *Use caution handling TCA and ether.*

Glutathione (GSH) standard:

First prepare a 4 mM GSH (5 mg GSH dissolved in 4 ml dH_2O). Dilute the solution 10-fold with dH_2O to obtain 0.4 mM (400 µM) GSH. Perform 1:1 serial dilution with dH_2O to obtain 200, 100, and 50 µM GSH standards. GSH standards should be prepared fresh for each assay.

Reagent thawing:

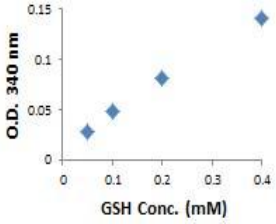
Keep thawed 200x CDNB at room temperature. Briefly vortex the CDNB vial to homogenize solution. Keep thawed 50x GST solution on ice (*do not over thaw GST solution*). Freeze the reagents immediately after use.

Preparation of working solution:

Each GSH standard and sample requires 0.2 ml working solution. Estimate the volume of working solution required for each experiment and prepare fresh. An example for 10 reactions: 2 ml GSH Assay Solution + 10 µl 200x CDNB. Vortex solution immediately to prevent CDNB precipitation. Proceed to add 40 µl 50x GST to the solution followed by brief mixing. Keep working solution on ice shielded from light and use immediately.

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. Alternatively, use a 0.2-ml quartz cuvette for the UV measurement.
2. Add 0.2 ml freshly prepared working solution to each well or tube. Mix contents by gentle agitation for 5 sec. Incubate at 37°C shielded from light for 20 min.
3. Measure $O.D._{340\ nm}$ using a plate reader or spectrophotometer. Presence of GSH will cause increased $O.D._{340\ nm}$ reading in a concentration-dependent manner.
4. Subtract blank reading from each GSH standard and sample reading to generate respective ΔO.D.. Generate a graph of GSH concentrations (x axis) vs. ΔO.D. (y axis) and a trendline equation.
5. Apply sample ΔO.D. to the equation to obtain sample GSH concentration. A new GSH standard plot must be generated for each assay.



Additional information:

- 200x CDNB contains dimethyl sulfoxide (DMSO) solvent and 1-chloro-2,4-dinitrobenzene. Please refer to the product page of our website or contact us for material SDS information.