

BIOMEDICAL RESEARCH SERVICE CENTER

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

COMPONENTS:
50x GST/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 a 0.2-ml Quartz cuvette or UV-transparent 96-well plate 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 dH₂O). Dilute the solution 10-fold with dH₂O 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 and 50x GST at room temperature and on ice, respectively. 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. If preparing sufficient working solution for 10 reactions, first add 40 μ l 50x GST/GSH Buffer to 2 ml dH₂O. Then add 10 μ l 200x CDNB and immediately vortex solution vigorously (initial cloudy appearance is normal). Finally add 40 μ l 50x GST followed by gentle vortexing. Keep working solution on ice and use as soon as possible.

ASSAY

1. Add 20 μ l dH₂O (blank), 20 μ l of each GSH standard and 20 μ l of each sample to 1.5-ml microtubes.
2. Add 0.2 ml freshly prepared working solution to each tube. Mix by brief vortexing and cap tubes. Incubate at 37°C for 20 min.
3. Measure O.D._{340 nm}. 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.

