EnzyChrom™ GSH/GSSG Assay Kit (EGTT-100)

Quantitative Determination of Total, Oxidized and Reduced Glutathione

DESCRIPTION

GLUTATHIONE, a tripeptide of glycine, glutamic acid and cysteine, is one of the key antioxidants involved in protecting cells from damages by reactive oxygen species. Glutathione (GSH) reduces disulfide bonds in cytoplasmic proteins to cysteines, in which it is converted to its oxidized form GSSG. BioAssay Systems' GSH/GSSG Assay Kit is designed to accurately measure total, reduced and oxidized glutathione in biological samples using an enzymatic method that utilizes Ellman's Reagent (DTNB) and glutathione reductase (GR). DTNB reacts with reduced glutathione to form a yellow product. The rate of change in the optical density, measured at 412 nm, is directly proportional to glutathione concentration in the sample. This kit can also be used to measure oxidized (GSSG) by using a specific protocol which first scavenges all GSH with 1-methyl-2-vinylpyridinium triflate.

KEY FEATURES

Sensitive and accurate. Linear detection range 0.01-3 µM GSH equivalents with a detection limit of 10 nM GSH equivalents.

APPLICATIONS

Direct Assays: total, reduced and oxidized glutathione in whole blood, plasma, serum, urine, tissue and cell extracts.
Drug Discovery/Pharmacology: effects of drugs on glutathione metabolism.

KIT CONTENTS (100 TESTS IN 96-WELL PLATES)

Scavenger: 500 µL **NADPH**: 40 µL **DTNB:** 60 µL
2× Assay Buffer: 25 mL **GR Enzyme**: 120 µL
Glutathione Standard: 50 µL

Note: *If planning to assay cell lysate, the kit only comes with sufficient Scavenger for 25 samples. If needed, additional vials of Scavenger can be ordered separately (Cat# EGTT-SCVG).*

Storage conditions. The kit is shipped on ice. Store all kit components at -20 °C. Shelf life of six months after receipt.

Precautions: reagents are for research use only. Normal precautions for laboratory reagents should be exercised while using the reagents. Please refer to Material Safety Data Sheet for detailed information.

PROCEDURES

Important: equilibrate Scavenger, DTNB and 2× Assay Buffer to room temperature before use. Dilute 2× Assay buffer with an equal volume of dH_2O to make 1× Assay Buffer. Briefly mix GR Enzyme before use. *Note*: β-mercaptoethanol, dithiothreitol and cysteine are known to interfere in this assay. Avoid using these compounds during sample preparation.

Sample Preparation for GSSG Measurement

Cell lysate can be prepared as follows: wash cells ($1\text{-}2 \times 10^6$) in cold PBS. Pellet cells by centrifugation at 300-900*g* for 5min. Remove PBS and lyse the cell pellet by homogenization or sonication in 200 µL of cold buffer containing 50 mM phosphate (pH = 7), 1 mM EDTA, and 20 µL Scavenger. Centrifuge at 10,000*g* for 5 min at 4°C. Transfer supernatant to a clean tube and proceed to the deproteination procedure.

Whole blood samples can be prepared as follows: mix 50 µL whole blood with 5 µL Scavenger and freeze at -70°C. (Freezing helps lyse the blood cells). After freezing, thaw and mix sample. Incubate at RT for 2-10 min then proceed to the deproteination procedure.

Sample Preparation for Total Glutathione Measurement

Cell lysate: wash and pellet cells (*see above*) in cold PBS. Lyse the cell pellet by homogenization or sonication in 200 µL of cold buffer containing 50 mM phosphate (pH = 7) and 1 mM EDTA (no Scavenger). Centrifuge at 10,000*g* for 15 min at 4°C. Transfer supernatant to a clean tube and proceed to the deproteination procedure.

Whole blood samples can be prepared as follows: freeze 50 µL whole blood at -70°C. (Freezing helps lyse the blood cells). After freezing, thaw and mix sample. Incubate at RT for 2-10 min then proceed to the deproteination procedure.

Deproteination Procedure.

Prepare a solution of 5wt% Metaphosphoric Acid (available separately at BioAssay Systems under cat# EGTT-MPA) in water (MPA Reagent). This reagent must be prepared fresh daily. Add 65 µL MPA Reagent to 25 µL sample, briefly vortex to mix and then centrifuge at 14000 rpm for 5 min. For total glutathione whole blood samples, transfer 5 µL of clear supernatant to a clean tube and mix with 620 µL 1× Assay Buffer. For all other samples, transfer 6 µL of clear supernatant to a clean tube and mix with 244 µL 1× Assay Buffer. Transfer 200 µL of each neutralized deproteinated sample to separate wells of a 96 well plate.

Glutathione Assay

1. *Standards*. First dilute GSH standard to 300 µM by mixing 3 µL 100 mM Standard with 997 µL dH_2O. Next, prepare the 3 µM Premix by mixing 5 µL of the 300 µM GSH with 495 µL 1× Assay Buffer. Dilute standards in 1.5-mL centrifuge tubes as described in the Table.

No	Premix + 1× Assay Buffer	GSH (µM)
1	250 µL + 0 µL	3.0
2	150 µL + 100 µL	1.8
3	75 µL + 175 µL	0.9
4	0 µL + 250 µL	0

Transfer 200 µL of each Standard to separate wells in a 96 well plate.

2. *Glutathione Detection*. Prepare enough working reagent (WR) for 4 standards and all samples. For each reaction combine the following: 105 µL 1× Assay Buffer, 1 µL GR Enzyme, 0.25 µL NADPH and 0.5 µL DTNB. Mix WR immediately after adding the DTNB. Add 100 µL of WR to each Standard and Sample well. Mix well.
3. Read OD_{412nm} at 0 min and again at 10 min.

CALCULATION

Subtract OD_{0min} from OD_{10min} for each Standard and sample. Next subtract the ΔOD_{BLANK} (Std 4) from the ΔOD values of all Standards and plot the ΔΔOD's against standard concentrations. Determine the slope using linear regression fitting. The GSSG and GSH concentrations of the Samples are calculated as follows:

$$[GSH_{TOTAL}] = \frac{\Delta OD_{SAMPLE} - \Delta OD_{BLANK}}{Slope} \times n \quad (\mu M)$$

$$[GSSG] = 0.5 \times \frac{\Delta OD_{S(GSSG)} - \Delta OD_{BLANK}}{Slope} \times n \quad (\mu M)$$

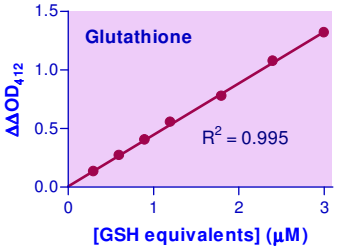
$$[GSH] = [GSH_{TOTAL}] - 2 \times [GSSG] \quad (\mu M)$$

ΔOD_{SAMPLE}, ΔOD_{BLANK} and $\Delta OD_{S(GSSG)}$ are the change in optical density values of the sample, water (Std 4) and sample *treated with Scavenger*, respectively. n is the dilution factor. For all samples treated with Scavenger, n = 165. For samples *not* treated with Scavenger, n = 450 for whole blood, and 150 for all other samples.
Conversions: 1 mg/dL glutathione equals 32.5 µM, 0.001% or 10 ppm.

MATERIALS REQUIRED, BUT NOT PROVIDED

Pipetting devices, clear flat-bottom 96-well plates, plate reader capable of reading optical density at 412 nm, centrifuge tubes and table centrifuge.

Metaphosphoric Acid can be purchased separately from BioAssay Systems (catalog#: MPA-2G) or Sigma-Aldrich (# 239275).



PUBLICATIONS

1. Hu Lee, J., et al. (2020). Epigenetic reprogramming of epithelial-mesenchymal transition promotes ferroptosis of head and neck cancer. Redox Biology 37: 101697.
2. Paul, S., et al. (2018). STAT3-RXR-Nrf2 activates systemic redox and energy homeostasis upon steep decline in pO2 gradient. Redox biology, 14, 423-438.
3. Lin, T. A., et al. (2019). Red Quinoa Bran Extracts Protects against Carbon Tetrachloride-Induced Liver Injury and Fibrosis in Mice via Activation of Antioxidative Enzyme Systems and Blocking TGF-beta1 Pathway. Nutrients, 11(2), 395.