Technical Manual

ChromaDazzle GSH/GSSG Assay Kit

Catalogue Code: BA0118

Pack Size: 100 assays

Research Use Only

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.

The Assay Genie ChromaDazzle 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
2X Assay Buffer:	25 mL	**GR Enzyme**:	120 µL		
Glutathione Standard:	50 µL				

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 2X Assay Buffer to room temperature before use. Dilute 2X Assay buffer with an equal volume of dH_2O to make 1X 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 (1-2 x 10^6) in cold PBS. Lyse cells 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 can be prepared as follows: wash cells (1-2 x 10^6) in cold PBS. Lyse cells by homogenization or sonication in 1 mL of cold buffer containing 50 mM phosphate (pH = 7) and 1 mM EDTA. 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 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 1X Assay Buffer. For all other samples, transfer 6 µL of clear supernatant to a clean tube and mix with 244 µL 1X 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 1X Assay Buffer. Dilute standards in 1.5-mL centrifuge tubes as described in the Table.

No	Premix + 1X 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 1X 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.

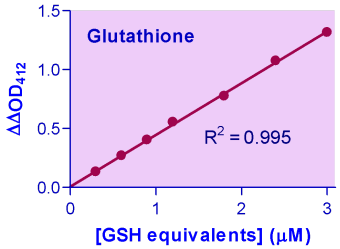
CALCULATION
Subtract OD_{0min} from OD_{10min} for each Standard and sample. Next subtract the DOD_{BLANK} (Std 4) from the DOD values of all Standards and plot the DDOD's against standard concentrations. Determine the slope using linear regression fitting. The GSSG and GSH concentrations of the Samples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

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

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

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

DOD_{SAMPLE}, DOD_{BLANK} and $DOD_{S(GSSG)}$ 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 multiple vendors including Sigma-Aldrich (# 239275).

LITERATURE

1. Hu XM, Hirano T, Oka K. (2003). Cancer Chemother Pharmacol 52:47-58.
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3. Katz A, Oldham KT, Guice KS, Coran AG. (1993). J Pediatr Surg. 28:1301-6.

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