QuantiChrom™ Glutathione Assay Kit (DIGT-250)

Colorimetric Determination of Reduced Glutathione at 412nm

DESCRIPTION

Glutathione is a tripeptide of glycine, glutamic acid and cysteine. In the red blood cell, the reduced form of glutathione is vital in maintaining hemoglobin in a reduced state and hence protecting the cells from oxidative damage. Glutathione is involved in detoxification of hydrogen peroxide through glutathione oxidase. Low levels of glutathione are found in deficiencies of key enzymes involved in glutathione metabolism, such as glucose-6-phosphate dehydrogenase, glutathione synthase and glutathione reductase.

Simple, direct and automation-ready procedures for measuring reduced glutathione are becoming popular in Research and Drug Discovery. BioAssay Systems' QuantiChrom™ Glutathione Assay Kit is designed to accurately measure reduced glutathione in biological samples. The improved 5,5'-dithiobis(2-nitrobenzoic acid (DTNB) method combines deproteination and detection into one reagent. DTNB reacts with reduced glutathione to form a yellow product. The optical density, measured at 412 nm, is directly proportional to glutathione concentration in the sample. The optimized formulation has a long shelf life and is completely free of interference due to sample turbidity.

KEY FEATURES

Sensitive and accurate. Linear detection range 0.4 - 100 µM in 96-well plate.
Simple and convenient. The procedure involves mixing the DTNB Reagent with sample, removing protein precipitates for proteinaceous samples, adding a second Reagent and reading the optical density.
Low interference. Amino acids and common buffers do not interfere.

APPLICATIONS:

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

KIT CONTENTS (250 tests in 96-well plates)

Reagent A: 30 mL Reagent B: 30 mL
Standard: 10 mL (= 100 µM glutathione)

Storage conditions. Store Reagent A and Reagent B at 4°C. The glutathione standard should be stored at -20°C. Shelf life: 12 months.

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 Reagents to room temperature before use.

Procedure using 96-well plate:

1. *Blank and Standard*. Transfer 100 µL water and 100 µL Standard into wells of a clear-bottom 96-well plate. Pipette 200 µL water into the Blank and Standard wells.
2. *Samples*. Whole blood samples should be diluted 20-fold with water prior to the assay (*n* = 20). Deproteination is required for blood, serum, plasma and other proteinaceous samples. Mix 120 µL sample with 120 µL Reagent A in 1.5-mL centrifuge tubes. Vortex to mix well. Pellet 2 min at 14,000 rpm in a table centrifuge. If solution remains clear, no centrifugation was necessary.
3. Transfer 200 µL sample/Reagent A mixture into wells of the 96-well plate. Add 100 µL Reagent B. Tap plate lightly to mix.
4. Incubate 20 to 25 min at room temperature. Read $OD_{400\text{-}450nm}$ (peak 412 nm). Signal is stable for at least 60 min.

Procedure using Cuvet:

Mix 400 µL sample with 400 µL Reagent A, centrifuge sample tubes if precipitation occurs. Transfer 600 µL supernatant and mix with 400 µL Reagent B. Incubate 25 min at room temperature. Measure OD_{412nm} against water. Transfer 400 µL Standard and 800 µL Water into a clean cuvet and measure OD_{412nm} against water.

CALCULATION

Subtract blank OD (water) from the Standard and Sample OD values. The glutathione concentration of Sample is calculated as

$$= \frac{OD_{SAMPLE} - OD_{BLANK}}{OD_{STD} - OD_{BLANK}} \times 100 \times n \ (\mu M)$$

OD_{SAMPLE}, OD_{STD} and OD_{BLANK} are optical density values of the sample, Standard and sample "Blank" (water or buffer in which the sample was dissolved). *n* is the dilution factor (20 for blood samples).
Conversions: 1 mg/dL glutathione equals 32.5 µM, 0.001% or 10 ppm.

MATERIALS REQUIRED, BUT NOT PROVIDED

Pipeting devices, centrifuge tube and table centrifuge.

Procedure using 96-well plate:

Clear bottom 96-well plates (e.g. Corning Costar) and plate reader.

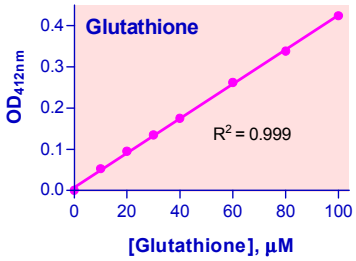
Procedure using cuvette:

Spectrophotometer and cuvets for measuring OD at 412 nm.

GENERAL CONSIDERATIONS

β-mercaptoethanol, dithiothreitol and cysteine are known to interfere in this assay. Avoid using these compounds during sample preparation. Amino acids do not interfere in this assay.

EXAMPLE. 20 µL fresh mouse blood was mixed quickly with 380 µL water. Assays in 96-well plate gave blood glutathione concentration of 1124 ± 8 µM (n = 2)



Calibration curve in 96-well plate

LITERATURE

1. Hu XM, Hirano T, Oka K. (2003). Arsenic trioxide induces apoptosis in cells of MOLT-4 and its daunorubicin-resistant cell line via depletion of intracellular glutathione, disruption of mitochondrial membrane potential and activation of caspase-3. Cancer Chemother Pharmacol 52:47-58.

2. Diebolt M, Bucher B, Andriantsitohaina R. (2001). Wine polyphenols decrease blood pressure, improve NO vasodilatation, and induce gene expression. Hypertension. 38:159-65.

3. Katz A, Oldham KT, Guice KS, Coran AG. (1993). Reperfusion injury following single-lung transplantation: the tissue glutathione response. J Pediatr Surg. 28:1301-6.