Technical Bulletin

Glutathione (GSH) Assay Kit

Catalogue number MAK517

Product 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 metabolomics research and drug discovery. The 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 (Reagent A) 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.

The linear detection range of the kit 0.4 - 100 µM. The kit is suitable for glutathione activity determination reduced glutathione in whole blood, plasma, serum, urine, tissue, and cell extracts.

Components

The kit is sufficient for 250 colorimetric assays in 96-well plates.

- Reagent A 30 mL
  Catalogue Number MAK517A
- Reagent B 30 mL
  Catalogue Number MAK517B
- Calibrator (equivalent to 100 µM glutathione) 10 mL
  Catalogue Number MAK517C

Reagents and Equipment Required but Not Provided

- Pipetting devices and accessories
- Spectrophotometric multiwell plate reader and cuvettes for procedure using cuvette.
- Clear flat-bottom 96-well plates. Cell culture or tissue culture treated plates are not recommended.
- Refrigerated microcentrifuge capable of RCF ≥10,000 × g.
- 1.5 mL microcentrifuge tubes
- Phosphate Buffered Saline (PBS) (Catalogue Number PPB006 or equivalent)

Precautions and Disclaimer

For R&D use only. Not for drug, household, or other uses. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.

Storage/Stability

The kit is shipped at room temperature. Store components at 2-8 °C.

Preparation Instructions

Briefly centrifuge small vials prior to opening. Equilibrate to room temperature prior use.

Reagent A: Shake well before use.

Procedure

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

Sample Preparation

All Samples and Standards should be run in duplicate.

Whole Blood

Dilute Samples 20-fold with purified water prior to the assay (DF = 20).

Cell Lysates

1. Centrifuge at 1000 *x g* for 10 minutes at 4 °C to collect 2 × 10^6 cells.
2. Wash cells in cold Phosphate Buffered Saline.
3. Lyse cells by homogenization or sonication in 1-2 mL of cold buffer containing 50 mM MES buffer (pH 6-7) containing 1 mM EDTA.
4. Centrifuge at 10,000 *x* g for 15 minutes at 4 °C. Use the supernatant for assay.

Note: Deproteination is required for blood, serum, plasma and other proteinaceous samples. Reagent A contains components for both color reaction and deproteination.

Assay Reaction

96-Well Plate Instructions

1. Transfer 100 µL water and 100 µL Calibrator into wells of a clear-bottom 96-well plate. Pipette 200 µL purified water into the Blank and Calibrator wells.
2. Mix 120 µL Sample with 120 µL Reagent A in 1.5 mL centrifuge tubes. Vortex to mix well. If turbidity occurs, pellet 5 minutes at 14,000 rpm in a centrifuge. If the mixture remains clear, no centrifugation is necessary.
3. Transfer 200 µL Sample/Reagent A mixture into wells of the 96-well plate.
4. Add 100 µL Reagent B. Tap plate lightly to mix.
5. Incubate at room temperature for 25 minutes.
6. Measure the Optical Density at 412 nm.

Cuvette Instructions

1. Mix 400 µL Sample with 400 µL Reagent A. Centrifuge Sample tubes if precipitation occurs.
2. Transfer 600 µL supernatant and mix with 400 µL Reagent B.
3. Incubate at room temperature for 25 minutes.
4. Measure the Optical Density at 412 nm against water.
5. Transfer 400 µL Calibrator and 800 µL water into a clean cuvet.
6. Measure the Optical Density at 412 nm against water.

Results

1. Subtract Blank OD from the Calibrator and Sample OD values.
2. The glutathione concentration of sample using the below equation:

$$\text{Glutathione } (\mu M) = \frac{OD_{Sample} - OD_{Blank}}{OD_{Calibrator} - OD_{Blank}} \times 100 \times DF$$

Where:

OD_{Sample} = Optical Density reading of the Sample

$OD_{Calibrator}$=Optical Density reading of the Calibrator

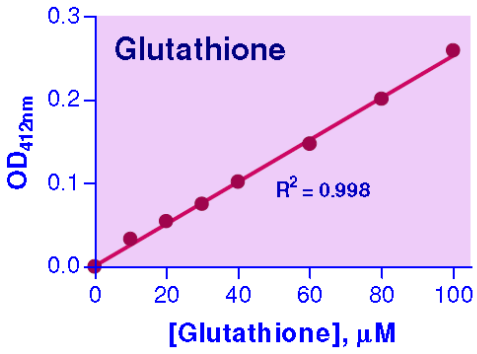
OD_{Blank} = Optical Density reading of the Sample Blank

DF = Dilution Factor (20 for blood samples).

Conversions: 1 mg/dL glutathione equals 32.5 µM, 0.001% or 10 ppm.

Figure 1.

Typical Glutathione Standard Curve in purified water.



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