Technical Bulletin

Glutathione GSH/GSSG Assay Kit

Catalog Number MAK440

Product Description

Glutathione, a tripeptide of glycine, glutamic acid, and cysteine, is one of the key antioxidants involved in protecting cells from damage by reactive oxygen species. Glutathione (GSH) reduces disulfide bonds in cytoplasmic proteins to cysteines, which results in glutathione being converted to its oxidized form GSSG.

The Glutathione GSH/GSSG Assay Kit is designed to accurately measure total, reduced (GSH) and oxidized (GSSG) 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 the glutathione concentration in the sample. This kit can also be used to measure oxidized glutathione (GSSG) by using a specific protocol which first scavenges all existing GSH using 1-methyl-2-vinylpyridinium triflate as a Scavenger reagent. The assay method has a linear detection range of 0.1 – 3 µM GSH equivalents, with a lower detection limit of 10 nM GSH equivalents.

The Glutathione GHS/GSSG Kit is suitable for the quantitative determination of reduced and oxidized glutathione (GSH/GSSG) in whole blood, plasma, serum, urine, and tissue and cell extracts.

Components

The kit is sufficient for 100 colorimetric assays in 96-well plates.

- Scavenger 500 µL
  Catalog Number MAK440A
- NADPH 40 µL
  Catalog Number MAK440B
- DTNB 60 µL
  Catalog Number MAK440C
- 2x Assay Buffer 25 mL
  Catalog Number MAK440D
- GR Enzyme 120 µL
  Catalog Number MAK440E
- Glutathione Standard 50 µL
  Catalog Number MAK440F

Reagents and Equipment Required but Not Provided

- Pipetting devices and accessories (e.g., multichannel pipettor)
- Spectrophotometric multiwell plate reader
- Clear flat-bottom 96-well plates. Cell culture or tissue culture treated plates are **not** recommended.
- 1.5 mL microcentrifuge tubes
- Dounce tissue grinder set (Catalog Number D9063 or equivalent)
- Refrigerated microcentrifuge capable of RCF ≥ 10,000 × *g*
- Phosphate Buffered Saline (PBS) (Catalog Number P3813 or equivalent)
- Sodium phosphate monobasic (Catalog Number S0751 or equivalent)

- EDTA disodium salt
  (Catalog Number ED2SS or equivalent)
- meta-Phosphoric acid
  (Catalog Number 239275 or equivalent)

Precautions and Disclaimer

For Research Use Only. Not for use in diagnostic procedures. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.

Storage/Stability

The kit is shipped on wet ice. Store components at -20 °C.

Preparation Instructions

Briefly centrifuge small vials prior to opening. Equilibrate Scavenger, DTNB and 2x Assay Buffer to room temperature prior to use.

1× Assay Buffer: Dilute 2x Assay buffer with an equal volume of purified water.

GR Enzyme: Briefly mix prior to use.

Procedure

All samples and standards should be run in duplicate.

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

Note: The volumes used for buffer and Scavenger should not be altered from the stated volumes.

1. Wash cells (1-2 × 10^6) in cold 1× PBS.
2. Centrifuge at 300-900 *x g* for 5 minutes at room temperature.
3. 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 of Scavenger.
4. Centrifuge at 10,000 × *g* for 5 minutes at 4 °C.
5. Transfer the supernatant to a clean tube and proceed to the deproteination procedure.

Whole Blood

1. Mix 50 µL of whole blood with 5 µL of Scavenger and freeze at -70 °C. Freezing helps to lyse the blood cells.
2. After freezing, thaw and mix sample.
3. Incubate at room temperature for 2-10 minutes, then proceed to the deproteination procedure.

Sample Preparation for Total Glutathione Measurement

Cell Lysate

Note: The volumes used for buffer and Scavenger should not be altered from the stated volumes.

1. Wash cells (1-2 × 10^6) in cold 1× PBS.
2. Centrifuge at 300-900 *x g* for 5 minutes at room temperature.
3. Remove PBS and lyse the cell pellet by homogenization or sonication in 200 µL of cold buffer containing 50 mM phosphate, pH 7, and 1 mM EDTA.
4. Centrifuge at 10,000 × *g* for 15 minutes at 4 °C.
5. Transfer the supernatant to a clean tube and proceed to the deproteination procedure.

Whole Blood

1. Freeze 50 µL of whole blood at -70 °C. Freezing helps to lyse the blood cells.
2. After freezing, thaw and mix sample.
3. Incubate at room temperature for 2-10 minutes, then proceed to the deproteination procedure.

Deproteination Procedure

1. Prepare a 5% (by weight) meta-Phosphoric Acid solution (MPA Reagent) in purified water. This reagent must be prepared fresh daily.
2. Add 65 µL of the MPA Reagent to 25 µL of sample/ Briefly vortex to mix and then centrifuge at 14,000 × *g* for 5 minutes.
3. For total glutathione whole blood samples, transfer 5 µL of clear supernatant to a clean tube and mix with 620 µL of 1× Assay Buffer.
4. For all other samples, transfer 6 µL of clear supernatant to a clean tube and mix with 244 µL of 1× Assay Buffer.
5. Transfer 200 µL of each neutralized deproteinated Sample from Step 3 or Step 4 to separate wells of a clear 96-well plate.

Glutathione Assay

Standard Curve Preparation

1. Prepare a 300 µM GSH Standard by mixing 3 µL of the 100 mM Glutathione Standard with 997 µL of purified water.
2. Prepare a 3 µM GSH Standard by mixing 5 µL of the 300 µM GSH Standard with 495 µL of 1× Assay Buffer.
3. Prepare GSH standards in 1.5 mL microcentrifuge tubes according to Table 1.

Table 1.
Preparation of GSH standards

Well	3 µM GSH Standard	1× Assay Buffer	GSH (µM)
1	250 µL	-	3.0
2	150 µL	100 µL	1.8
3	75 µL	175 µL	0.9
4	-	250 µL	0

4. Mix well and transfer 200 µL of each Standard into separate wells of the plate.

Working Reagent

1. Mix enough reagent for the number of assays to be performed. For each Standard and Sample well, prepare 106.75 µL of Working Reagent according to Table 2.

Table 2.
Preparation of Working Reagent

Reagent	Working Reagent
1× Assay Buffer	105 µL
GR Enzyme	1 µL
NADPH	0.25 µL
DTNB	0.5 µL

2. Mix the Working Reagent immediately after adding the DTNB reagent.

Measurement

1. Add 100 µL of the Working Reagent to each Sample and Standard well. Tap plate to mix.
2. Read the optical density (OD) of each well at 412 nm at zero and 10 minutes (OD_{0Min} and OD_{10Min} respectively).

Results

1. Calculate the ΔOD by subtracting the OD_{0Min} reading from the OD_{10Min} reading for each Standard and Sample.
2. Subtract the ΔOD_{Blank} (Standard #4) from the remaining Standards. Plot the corrected ΔOD against the standard concentrations.
3. Determine the slope of the standard curve using linear regression.
4. Calculate the GSSG and GSH concentrations of the sample:

$$GSH_{Total}\ (\mu M) = \frac{\Delta OD_{Sample} - \Delta OD_{Blank}}{\text{Slope } (\mu M^{-1})} \times DF$$

$$GSSG\ (\mu M) = 0.5 \times \frac{\Delta OD_{S(GSSG)} - \Delta OD_{Blank}}{\text{Slope } (\mu M^{-1})} \times DF$$

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

where

ΔOD_{Sample} = Change in OD values of Sample between zero minutes and 10 minutes.

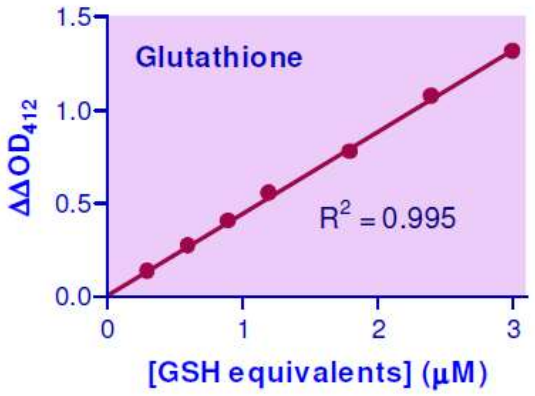
ΔOD_{Blank} = Change in OD values of Blank (Standard #4) between zero minutes and 10 minutes.

$\Delta OD_{S(GSSG)}$ = Change in OD values of Sample treated with Scavenger between zero minutes and 10 minutes. See Sample Preparation for GSSG Measurement.

DF = For all samples treated with Scavenger, DF = 165. For samples not treated with Scavenger, DF = 450 for whole blood, and 150 for all other samples.

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

Figure 1.
Typical Glutathione Standard Curve



References

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3. Lin, T.A., et al., 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 (2019).

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