Technical Bulletin

Glutathione Reductase Kit

Catalogue number MAK535

Product Description

Glutathione Reductase (GR) reduces oxidized glutathione (GSSG) to the reduced sulfhydryl form GSH which is an important cellular antioxidant. A high GSH/GSSG ratio is important for protection against oxidative stress. Thus, measurement of GR activity is used as indicator for oxidative stress. The non-radioactive colorimetric GR assay is designed to accurately measure GR activity in biological samples with a method that utilizes Ellman's method in which DTNB reacts with the GSH generated from the reduction of GSSG by the GR in a sample to form a yellow product (TNB2-). The rate of change in the optical density, measured at 412 nm, is directly proportional to the Glutathione Reductase activity in the sample.

The linear detection range of the kit is 0.4 to 50 U/L. The kit is suitable for Glutathione Reductase determination in biological samples such as plasma, serum, tissue, and culture media.

Components

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

- Assay Buffer 10 mL
  Catalogue Number MAK535A
- GDH 120 µL
  Catalogue Number MAK535B
- Substrate 1 mL
  Catalogue Number MAK535C
- DTNB 60 µL
  Catalogue Number MAK535D
- Cosubstrate 1 mL
  Catalogue Number MAK535E
- Calibrator 1.5 mL
  Catalogue Number MAK535F

Reagents and Equipment Required but Not Provided

- Pipetting devices and accessories (example., 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 centrifuge tubes
- Dounce tissue grinder set.
  (Catalogue Number D9063 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.

Assays can be executed at any desired temperature 25 °C or 37 °C, equilibrate all components to desired temperature prior to use.

Procedure

All Samples and Standards should be run in duplicate.

Sample Preparation

Serum and plasma are assayed directly.

Tissue

1. Prior to dissection, rinse tissue in phosphate buffered saline (pH 7.4) to remove blood.
2. Homogenize 50 mg of tissue in ~ 200 µL of buffer containing 50 mM potassium phosphate (pH 7.5)
3. Centrifuge at 10,000 × g for 15 minutes at 4 °C.
4. Remove supernatant for assay.

Cell Lysate

1. Collect cells by centrifugation at 2,000 × g for 5 minutes at 4 °C.
2. For adherent cells, do not harvest cells using proteolytic enzymes. Instead, use a rubber policeman.
3. Homogenize or sonicate cells in an appropriate volume of cold buffer containing 50 mM potassium phosphate (pH 7.5)
4. Centrifuge at 10,000 × g for 15 minutes at 4 °C.
5. Remove supernatant for assay.

All Samples can be stored at -20 °C to -80 °C for at least one month.

Working Reagent

For each well, prepare 87.5 µL of Working Reagent according to Table 1.

Table 1.
Preparation of Working Reagent

Reagent	Volume
Substrate	8 µL
Cosubstrate	8 µL L
GDH	1 µL
DTNB	0.5 µL
Assay Buffer	70 µL

Assay Reaction

1. Transfer 100 µL of Calibrator and 100 µL Assay Buffer to separate wells.
2. Transfer 20 µL of each Sample into separate wells.
3. Transfer 80 µL of working reagent to each Sample well. Tap plate briefly to mix.
4. Incubate the plate at desired temperature for 10 minutes.

Note: This assay is based on a kinetic reaction. To ensure identical incubation time, addition of Working Reagent to Samples should be quick and mixing should be brief but thorough. Use of a multi-channel pipettor is recommended.

Measurement

Read the optical density (OD) of at 412 nm and again after 30 minutes (OD_{10} and OD_{30} respectively). Alternatively, using the plate reader's kinetic mode, monitor the OD for 30 minutes.

Results

1. Subtract the OD_{10} from OD_{30} for each sample to compute the ΔOD_S.
2. Glutathione Reductase activity can then be calculated as follows:

GR Activity =

$$\frac{\Delta OD_S}{2\times\varepsilon_{TNB} \times l} \times \frac{\text{Reaction Vol (µL)}}{\text{t (min)} \times \text{Sample Vol (µL)}} \times n$$

$$= \frac{\Delta OD_S}{OD_{Cal} - OD_{Buffer}} \times \frac{440}{\text{t (min)}} \times n \text{ (U/L)}$$

Where:

ε_{TNB} = Molar absorption coefficient of TNB generated for each mole of GSSG converted by GR

l = Light pathlength which is calculated from the calibrator

OD_{Cal} and OD_{Buffer} are OD_{412nm} values of the Calibrator and Assay Buffer

t = Reaction time (20 min)

Reaction Vol = 100 µL

Sample Vol = 20 µL

n = Dilution factor

Note: If sample GR activity exceeds 50 U/L, either use a shorter reaction time or dilute samples in water and repeat the assay.

Unit definition: 1 Unit (U) of GR will catalyze the conversion of 1 µmole of GSSG to 2 µmole GSH per min at pH 7.6.

Figure 1.
Example of Raw Kinetics Data

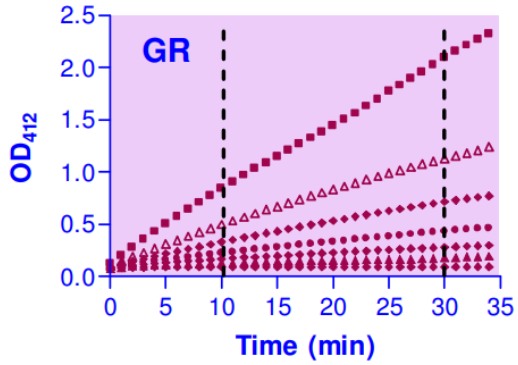
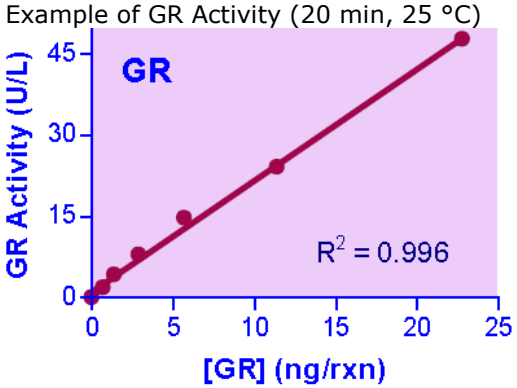


Figure 2.
Example of GR Activity (20 min, 25 °C)



References

1. Delides, A *et. al.,* An optimized semi-automatic rate method for serum glutathione reductase activity and its application to patients with malignant disease. *J. Clin. Path.,* **29**, 73-7 (1976).
2. Smith, IK *et. al.,* Assay of glutathione reductase in crude tissue homogenates using 5,5'-dithiobis(2-nitrobenzoic acid). *Anal. Biochem.,* **175**, 408-13 (1988).
3. Cribb, AR *et. al.,* Use of a microplate reader in an assay of glutathione reductase using 5,5'-dithiobis(2-nitrobenzoic acid). *Anal. Biochem.,* **183**, 195-6. (1989).

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