Glutathione Reductase Assay Kit

Glutathione Reductase Assay Kit is a detection kit for the quantification of Glutathione Reductase activity in serum, plasma, tissue and cell culture supernatants.

Catalog number: ARG82119

Package: 100 tests

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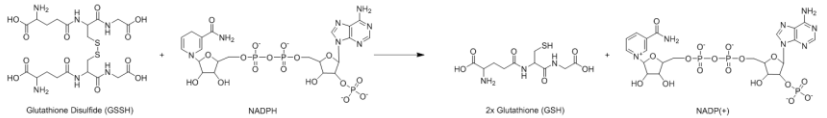
INTRODUCTION

Glutathione reductase (GR) also known as glutathione-disulfide reductase (GSR) is an enzyme that in humans is encoded by the GSR gene. Glutathione reductase (EC 1.8.1.7) catalyzes the reduction of glutathione disulfide (GSSG) to the sulfhydryl form glutathione (GSH), which is a critical molecule in resisting oxidative stress and maintaining the reducing environment of the cell. Glutathione reductase functions as dimeric disulfide oxidoreductase and utilizes an FAD prosthetic group and NADPH to reduce one molar equivalent of GSSG to two molar equivalents of GSH:



The glutathione reductase is conserved between all kingdoms. In bacteria, yeasts, and animals, one glutathione reductase gene is found; however, in plant genomes, two GR genes are encoded. Drosophila and trypanosomes do not have any GR at all. In these organisms, glutathione reduction is performed by either the thioredoxin or the trypanothione system, respectively. [Provide by Wikipedia: Glutathione reductase]

PRINCIPLE OF THE ASSAY

This Glutathione Reductase Assay Kit is a simple colorimetric assay that measures the amount of Glutathione Reductase (GR) present in serum, plasma, tissue and cell culture supernatants. The assay is initiated 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 samples to form a yellow product (TNB^{2-}). The rate of change in the optical density, measured at 412 nm, is directly proportional to GR activity in the sample. The concentration of GR in the samples is then determined by comparing the O.D. 412 nm absorbance of samples to the standard curve.

MATERIALS PROVIDED & STORAGE INFORMATION

The kit is shipped on ice. Store all components at -20°C upon receiving. Shelf life: 6 months after receipt.

Component	Quantity	Storage information
Assay Buffer	10 mL	-20°C
Substrate	1 mL	-20°C
Cosubstrate	1 mL	-20°C
GDH	120 µL	-20°C
DTNB	60 µL	-20°C
Standards	1.5 mL	-20°C

MATERIALS REQUIRED BUT NOT PROVIDED

- Microplate reader capable of reading at O.D. 412 nm
- Centrifuge
- Mortar
- Clear flat-bottom 96 well plate
- Deionized or Distilled water
- 1X PBS, pH 7.4
- Ice
- Pipettes, pipette tips and Multichannel micropipette reservoir

TECHNICAL NOTES AND PRECAUTIONS

- Wear protective gloves, clothing, eye, and face protection especially while handling blood or body fluid samples.
- 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.
- All reagents should be mixed by gentle inversion or swirling prior to use. Do not induce foaming.
- Before using the kit, spin tubes and bring down all components to the bottom of tubes.
- It is highly recommended assaying the Standards and samples in duplicates.
- Change pipette tips between the addition of different reagent or samples.

SAMPLE COLLECTION & STORAGE INFORMATION

The sample collection and storage conditions listed below are intended as general guidelines. Sample stability has not been evaluated.

Cell lysate samples: Collect cells by centrifugation at 2,000 x g for 5 min at 4°C. For adherent cells, do not harvest cells using proteolytic enzymes; rather use a rubber policeman. Homogenize or sonicate cells in an appropriate volume of 1X PBS. Centrifuge at 10,000 x g for 15 min at 4°C. Take supernatant for assay.

Tissue samples: Weigh out 50 mg tissue, homogenize with 0.2 mL of 1X PBS on ice, centrifuged at 10,000 x g for 15 minutes at 4°C. Take the supernatant into a new centrifuge tube and keep it on ice for detection.

Serum: Collect blood in a tube with no anticoagulant. Allow the blood to clot at room temperature for 30 minutes. Centrifuge at 2500 x g for 20 minutes at 4°C.

Plasma: Collect blood with EDTA, heparin or citrate and centrifuge at 2000 x g for 10 minutes at 4°C. Collect the plasma layer and store on ice.

Note:

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

REAGENT PREPARATION

- **Working Reagent**: add 8 µL of Substrate, 8 µL of Cosubstrate, 1 µL of GDH, 0.5 µL of DTNB and 70 µL of Assay Buffer. (For 96 well assay)

ASSAY PROCEDURE

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. Assays can be executed at any desired temperature (E.g. 25°C or 37°C).

1. Transfer **100 µL** of **Standard** and **100 µL** of **Assay Buffer** to separate wells in a **96 well microplate**.
2. Transfer **20 µL** of each **sample** into wells and add **80 µL** of **Working Reagent** to each sample well. Tap plate briefly to mix.
3. Incubate for **10 minutes** and read the absorbance at **O.D. 412 nm** (OD_{10}), and again after **30 minutes** (OD_{30}) on a microplate reader.

CALCULATION OF RESULTS

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

   GR Activity (U/L) = (ΔOD_S / 2 x ε_{TNB} x *l*) x (Reaction Vol / t x Sample Vol) x n

   = (440 / t) x (ΔOD_S / $OD_{Standard}$ – OD_{Buffer}) x n

   Note:

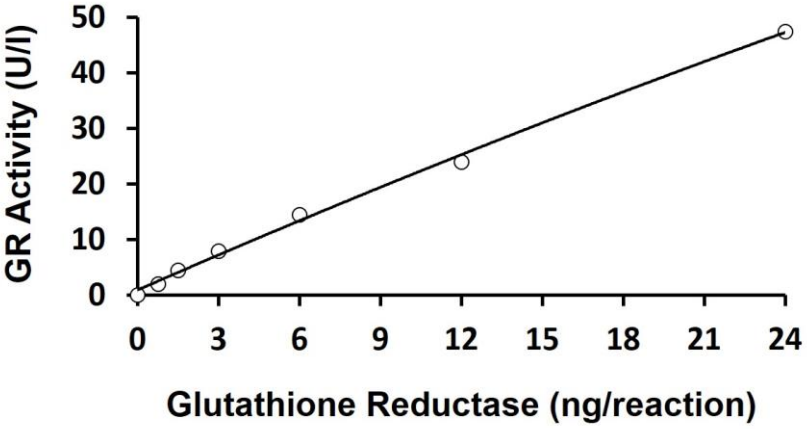
   - ε_{TNB}: molar absorption coefficient of TNB
   - 2: the number of moles of TNB generated for each mole of GSSG converted by GR.
   - *l*: the light pathlength which is calculated from the calibrator.
   - $OD_{Standard}$: O.D. 412 nm value of the Standard
   - OD_{Buffer}: O.D. 412 nm value of the Assay Buffer
   - t: reaction time (20 minutes is the recommended time)
   - Reaction Vol: 100 µL
   - Sample Vol: 20 µL
   - N: dilution factor

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

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

EXAMPLE OF TYPICAL STANDARD CURVE

The following figures demonstrate typical results with the Glutathione Reductase Assay Kit. One should use the data below for reference only. This data should not be used to interpret actual results.



QUALITY ASSURANCE

Sensitivity

0.4 U/L