MANUAL

Glutathione Reductase (GR) Activity Assay Kit (Colorimetric)

Catalog #: MA-GR

RayBiotech
Empowering your proteomics

ISO 13485:2016

Introduction

Glutathione reductase is a homodimeric enzyme that belongs to the flavoprotein disulfide oxidoreductases family. It plays an indirect role in the prevention of oxidative damage in cells by maintaining intracellular reduced glutathione (GSH). Thus, measuring the activity of this enzyme is an indicator of oxidative stress. It is a ubiquitous enzyme that catalyzes the NADPH dependent reduction reaction of oxidized glutathione (GSSG) to reduced glutathione (GSH). Maintaining GSH is vital for oxidation-reduction processes and for detoxifying hydrogen peroxide and organic peroxides produced by inflammation in cells.

Glutathione Reductase (GR) Activity Assay Kit offers a user-friendly, reliable, and highly sensitive method for quantifying GR activity in plasma, serum, cell lysates, and other biological fluid samples. In this assay, Glutathione Reductase reduces oxidized glutathione (GSSG) to reduced glutathione (GSH) in the presence of NADPH. The oxidation of NADPH to $NADP^+$ is accompanied by a decrease in absorbance at 340nm. The rate of decrease in the absorbance is directly proportional to the GR activity in the sample.

Storage

The entire kit may be stored below -20 °C for up to 6 months from the date of shipment. For prepared reagent storage, see table below.

Component	Size / Description	Storage After Preparation
Microplate	A 96-well (12 strips x 8 wells) plate	RT*
Assay Buffer	20 ml	2–8 °C, 6 months
GSSG	4 Vials (Lyophilized)	Use immediately
NADPH	2 Vials (Lyophilized)	Use immediately
Glutathione Reductase (GR) Positive Control	1 Vial (Lyophilized)	Prepare aliquots, store at -20 °C. Avoid multiple freeze-thaw cycles.

**RT = room temperature*

Additional Materials Required

1. Microplate reader capable of measuring absorbance at 340 nm at 25°C
2. Precision pipettes to deliver 2 µl to 1 ml volumes
3. Multi-channel pipettes to deliver 20 µl to 200 µl volumes
4. Tubes to prepare sample dilutions
5. 15 ml conical tubes
6. Incubator at 25ºC
7. Deionized water

Sample Tips and General Considerations

NOTE: Optimal methods of sample preparation will need to be determined by each researcher empirically based on researched literature and knowledge of the samples.

- If not using fresh samples, freeze samples as soon as possible after collection.
- Avoid multiple freeze-thaw cycles. If possible, sub-aliquot samples prior to initial storage.
- It is strongly recommended to add a protease inhibitor cocktail to cell and tissue lysate samples.
- Avoid sonication of 1 ml or less as this can quickly heat and denature proteins.
- Most samples will not need to be concentrated. If concentration is required, a spin column concentrator with a chilled centrifuge is recommended.

1. Cell lysates can be prepared as follows:

For attached cells, remove supernatant from cell culture, wash cells twice with cold 1X PBS (for suspension cells, pellet the cells by spinning down the cells at 1,000 x g for 10 min) making sure to remove any remaining PBS before adding lysis buffer. Solubilize the cells at $2x10^7$ cells/ml in lysis buffer containing protease inhibitors. Pipette up and down to resuspend cells and rock the lysates gently at 2–8 °C for 30 minutes. Transfer extracts to microfuge tubes and centrifuge at 14,000 x g for 10 minutes.
It is recommended that sample protein concentrations should be determined using a total protein assay. Lysates should be used immediately or aliquot and stored at -70 °C. Thawed lysates should be kept on ice prior to use.
General tips for preparing lysate samples can be viewed on the online Resources page of the website: https://www.raybiotech.com/tips-on-sample-preparation/

2. Plasma samples:

Collect blood with an anticoagulant such as citrate, EDTA or oxalate and mix by inversion. Centrifuge the blood at 1000 x g at 4°C for 10 minutes. Collect plasma supernatant without disturbing the white buffy layer. Sample should be tested immediately or frozen at -80°C for storage. Plasma sample can be loaded neat. Use Assay buffer to dilute if needed.

3. Serum samples:

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. Remove the yellow serum supernatant without disturbing the white buffy layer. Samples should be tested immediately or frozen at -80°C for storage. Serum sample can be loaded neat. Use Assay buffer to dilute if needed.

NOTE:
If the calculated GR activity of the sample is higher than 150 U/L, dilute the sample in Assay Buffer and repeat the assay.

Reagent Preparation

A. Working Solution

1. GSSG: Briefly spin down the vial, add 600 µL assay buffer to each vial. Mix well.
2. **Working Solution**: Add 1100 µL of reconstituted GSSG into 4400 µL of assay buffer to make Working Solution enough for half plate. Mix well.

B. Positive Control

Glutathione Reductase (GR) Positive Control: Briefly spin down the vial, add 250 µL Assay Buffer to the vial to create GR stock. Mix well. Then add 50 µL of GR stock into 50 µL Assay Buffer to make GR Positive Control.

C. NADPH Solution

1. NADPH: Briefly spin down the vial, add 240 µL deionized water to each vial to make 10X NADPH Stock.
2. **NADPH solution**: Add 220 µL of 10X NADPH Stock into 1980 µL of assay buffer to make enough NADPH Solution for half a plate. Mix well. Protect from light. Keep at 4 ^{o}C. The reconstituted reagent should be used within 4 hours.

Assay Procedure

Positive control, sample and Assay Buffer (used as a blank) should be assayed in duplicate or triplicate. A freshly prepared positive control should be used each time the assay is performed.

1. Prepare Working Solution (See Reagent Preparation, section A).
2. Prepare Positive Control (See Reagent Preparation, section B).
3. Prepare NADPH Solution (See Reagent Preparation, section C).
4. Add 20 µL of the diluted GR Positive Control, Assay Buffer (used as a blank) or unknown samples to the 96-well microtiter plate.
5. Add 100 µL of Working Solution to each well. Mix thoroughly. Incubate for 5 min at 25 ^{o}C with gentle shaking.
6. After five minutes, initiate the reaction by adding 40 µL of the NADPH Solution into each well (it is recommended to use a multi-channel pipette) and mix thoroughly.
7. Read and record absorbance at 340nm every minute for 5 time points at 25 ^{o}C.

Calculation of Results

Calculate the mean absorbance for each set of duplicate/triplicate sample, Positive Control, and Assay Buffer blank.

Calculate the average absorbance difference per minute ΔOD_{340nm}/min.

$$\Delta OD_{340nm}/min = \frac{(A1-A2) + (A2-A3) + (A3-A4) + (A4-A5)}{4}$$

Subtract the absorbance difference per minute of the Blank wells from absorbance difference per minute of the sample and positive control, this is the corrected absorbance difference per minute.

$$\Delta OD'_{340nm}/min = \Delta OD_{340nm}/min - \Delta OD^{Blank}_{340nm}/min$$

One international Unit (U/L) is defined as the amount of enzyme that catalyzes the transformation of one micromole of substrate per minute.

$$GR\ activity\ (U/L) = \frac{\Delta OD'_{340nm}/min \times 160\ \mu l \times 1000}{2.689\ mM^{-1} \times 20\ \mu l} \times sample\ dilution$$

Where: $\Delta OD'_{340nm}$ */min* = Corrected absorbance change per minute

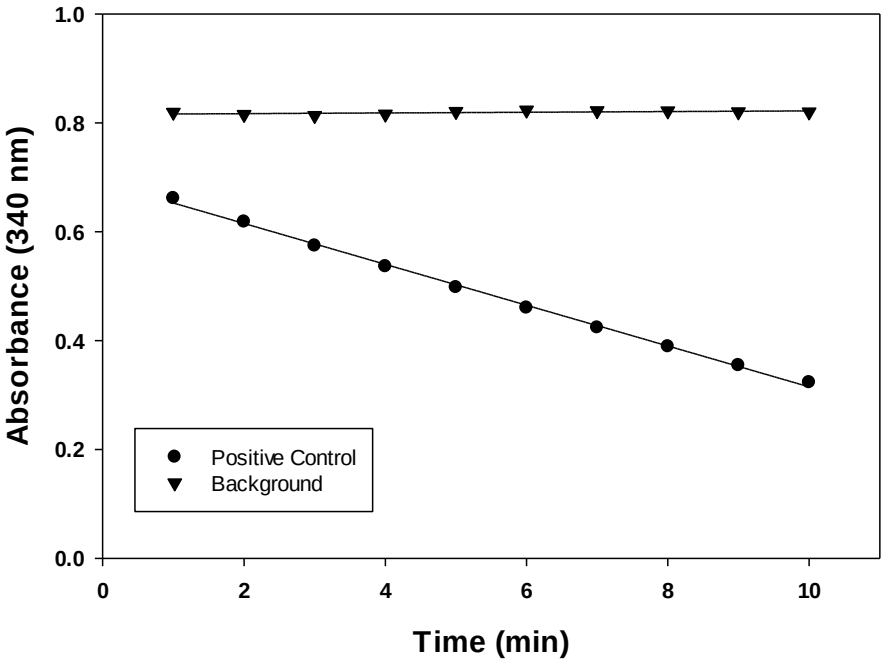
1000 = Conversion of U/ml to U/L

2.689 mM^{-1} is the adjusted extinction coefficient for NADH at 340nm with the current path length of the solution in a 96-well plate.

*GR activity International System of Units (SI Units) (nkat/L) = GR activity (U/L)*16.67*

Note: If the calculated GR activity of the sample is higher than 150 U/L, dilute the sample in Assay Buffer and repeat the assay.

A. Typical Data



Example of OD value of Glutathione Reductase (GR) Positive Control verse Time. This is an example. Results will vary.

B. Linear Range

3.6 U/L to 150 U/L GR activity.

C. Reproducibility

Intra-assay Precision (Precision within an assay):
To assess intra-assay precision, 16 wells per sample (total of 4 samples) were tested on a single plate. The intra-assay coefficient of variation was found to be 3.6%.

Inter-assay Precision (Precision between assays):
To assess inter-assay precision, 4 samples were tested in separate assays (n=4). The inter-assay coefficient of variation was found to be 6.1%.

This product is for research use only.