Glutathione Reductase Assay Kit

Catalog No.: abx298809

Size: 100 tests

Detection Range: 6.2 U/L – 320 U/L

Sensitivity: 6.2 U/L

Storage: Store Buffer solution at 4°C, and all other components at -20°C.

Application: For detection and quantification of GSH-PX activity in serum, plasma, tissue, cell samples, and other biological fluids.

Introduction

Glutathione Reductase (GR) is an enzyme that catalysed the reduction of glutathione disulfide to the sulfhydryl form of glutathione (GSH), which is a critical antioxidant. GR is a ubiquitous enzyme, with redox activity that is essential to maintaining the reducing environment of cells.

Abbexa's GR Assay Kit is a quick, convenient, and sensitive method for measuring and calculating GR activity. The absorbance should be measured at 340 nm. The intensity of the color is proportional to the activity of the GR enzyme(s), which can then be calculated.

Kit components

1. Buffer solution: 6 × 60 ml
2. Substrate: 8 vials
3. Enzyme: 4 vials

Materials Required But Not Provided

1. Spectrophotometer (340 nm) and 1 cm optical path cuvettes
2. Double distilled water
3. Normal saline (0.9% NaCl) or PBS (0.01 M, pH 7.4)
4. Homogenization medium (PBS 0.01 M, pH 7.4; 0.1 mM EDTA; 1.5% KCl)
5. Pipette and pipette tips
6. Vials/tubes
7. Incubator
8. Centrifuge
9. Vortex mixer

Protocol

A. Preparation of samples and reagents

1. Samples

Isolate the test samples soon after collecting and analyze immediately or aliquot and store at -20°C or -80°C for long term storage. Avoid multiple freeze-thaw cycles.

The following sample preparation methods are intended as a guide and may be adjusted as required depending on the specific samples used.

- **Serum:** Samples should be collected into a serum separator tube. Coagulate the serum by leaving the tube undisturbed in a vertical position overnight at 4°C or at room temperature for up to 1 hr. Centrifuge at approximately 2000 × g for 15 mins at 4°C. If a precipitate appears, centrifuge again. Take the supernatant, keep on ice and assay immediately, or aliquot and store at -80°C for up to 1 month.
- **Plasma:** Collect plasma using heparin as the anticoagulant. Centrifuge for 10 mins at 1000-2000 × g at 4°C, within 30 mins of collection. If precipitate appears, centrifuge again. Avoid hemolytic samples. Take the supernatant (avoid taking the middle layer containing white blood cells and platelets), keep on ice and assay immediately, or aliquot and store at -80°C for up to 1 month.
- **Tissue Homogenates:** Weigh the tissue homogenate. For each 1 g of homogenate, add 9 ml homogenization medium (PBS 0.01 M, pH 7.4; 0.1 mM EDTA; 1.5% KCl). Homogenize by hand, using a mechanical homogenizer, or by ultrasonication. Centrifuge the homogenate at 10,000 x g at 4°C for 10 min. Collect the supernatant and assay immediately. The protein concentration in the supernatant should be determined separately.
- **Cell lysates:** Collect cells into a centrifuge tube and wash with PBS. Centrifuge at 1000 × g for 10 min and discard the supernatant. Add 300-500 µl homogenization medium (PBS 0.01 M, pH 7.4; 0.1 mM EDTA; 1.5% KCl) per 1 × 10^6 cells, then sonicate in an ice water bath. Centrifuge at 1500 × g at 4 °C for 10 min. Take the supernatant into a new centrifuge tube (while kept on ice) and analyze immediately. The protein concentration in the supernatant should be determined separately.

Samples should not contain detergents such as SDS Tween-20, NP-40 and Triton X-100, or reducing agents such as DTT.

We recommend carrying out a preliminary experiment to determine the optimal dilution factor of samples before carrying out the formal experiment.

Note:

- Fresh samples or recently obtained samples are recommended to prevent degradation and denaturalization that may lead to erroneous results.
- Lysis buffers may interfere with the kit. It is therefore recommended to use mechanical lysis methods for cell lysates and tissue homogenates.
- Test one sample at a time. The timing of the assay procedures should be accurate.

The following sample dilutions are intended as a guide, the optimal dilution factor should be determined by the end user. Dilute using Double distilled water; or Normal saline, 0.9% NaCl.

Sample	Dilution factor
Human Serum	1
Human Plasma	1
Mouse Serum	1
Mouse Plasma	1
Rat Serum	1
Rat Plasma	1
10% Mouse liver tissue homogenate	1-3
10% Epipremnum aureum tissue homogenate	1

2. Reagents

- **Substrate solution:** Dissolve a vial of Substrate with 1 ml of Double distilled water and mix fully. The resulting solution can be stored at 4°C for up to 2 days.
- **Enzyme solution:** Dissolve a vial of Enzyme with 1 ml of Double distilled water and mix fully. The resulting solution can be stored at -20°C for up to 2 days.
- **Assay working solution:** Mix Buffer solution, Substrate solution and Enzyme solution at a ratio of 230 : 6 : 3. For example, mix 6900 µl of Buffer solution, 180 µl of Substrate solution and 90 µl of Enzyme solution to produce 7170 µl of Assay working solution. Prepare the required volume before use. The Assay working solution can be stored at 4°C for up to 4 days

B. Assay Procedure

1. Prepare sample, blank and zero cuvettes. Preheat the cuvettes in an incubator at 37°C for 5 minutes. *The reaction in this assay is temperature sensitive. Cuvettes must be at 37°C when absorbance is being measured.*
2. Set the spectrophotometer to 340 nm. Zero the spectrophotometer with double distilled water.
3. Add 65 µl of Double distilled water and 3120 µl of Assay working solution to the blank cuvette and mix fully.
4. Add 65 µl of sample into the sample cuvette. Add 3120 µl of Assay working solution, mix immediately and begin the timer.
5. Incubate sample and blank cuvettes at 37°C. Measure the absorbance at 340 nm at 30 seconds (A_1) and 150 seconds (A_2)

C. Calculation of Results

The standard curve can be plotted as the absolute OD_{340} of each standard solution (*y*) vs. the respective concentration of the standard solution (*x*). A linear fit is recommended for the standard curve ($y = ax + b$). The GSH-PX concentration of the samples can be interpolated from the standard curve.

1. Serum, plasma and cell culture supernatant samples:

One unit of GSH-PX activity is defined as the quantity of GR in 1 ml of sample that catalyzes the consumption of 1 mmol NADPH 37°C over 2 minutes.

$$\mathrm{GR\ (U/L)} = \frac{\Delta A_{\mathrm{sample}} - \Delta A_{\mathrm{blank}}}{\varepsilon \times 1} \times \frac{V_1}{t \times V_2} \times f$$

2. Tissues and cell lysate samples:

One unit of GSH-PX activity is defined as the quantity of GR in 1 g of protein that catalyzes the consumption of 1 mmol NADPH at 37°C over 2 minutes.

$$\mathrm{GR\ (U/g\ prot)} = \frac{\Delta A_{\mathrm{sample}} - \Delta A_{\mathrm{blank}}}{\varepsilon \times 1} \times \frac{V_1}{t \times V_2 \times C_P} \times f$$

where:

GR	activity of glutathione reductase
$\Delta A_{\mathrm{Sample}}$	change in OD value of the cuvette between 30 s and 150 s ($\Delta A_{1\ (\mathrm{sample})} - \Delta A_{2\ (\mathrm{sample})}$)
$\Delta A_{\mathrm{Blank}}$	change in OD value of the blank cuvette between 30 s and 150 s ($\Delta A_{1\ (\mathrm{blank})} - \Delta A_{2\ (\mathrm{blank})}$)
ε	molar extinction coefficient of 1 mM NADPH 340 nm, 1 cm optical path : 6.22 L/mmolcm
f	dilution factor of the sample
V_1	volume of sample in the definition (1 L = 1000 ml)
V_2	volume of sample added to the reaction (0.065 ml)
1	optical path (1 cm)
t	reaction time, 2 minutes
C_P	concentration of protein in sample (g protein/L)