

Technical Bulletin

Glutathione Reductase Assay Kit

Catalogue number GRSA

Product Description

Glutathione reductase (GR, EC 1.6.4.2) catalyzes the reduction of oxidized glutathione (GSSG) to reduced glutathione (GSH). This enzyme, which is found in many tissues, enables the cell to sustain adequate levels of cellular GSH. Reduced glutathione is a substrate for the glutathione peroxidases, which provide a mechanism for the detoxification of peroxides, and glutathione S-transferases, which are involved in the conjugation and elimination of xenobiotics from the organism. Reduced glutathione also acts as an antioxidant, reacting with free radicals and organic peroxides.

The Glutathione Reductase Assay Kit enables the spectrophotometric measurement of glutathione reductase activity. The activity can be measured by either the decrease in absorbance caused by the oxidation of NADPH at 340 nm (UV assay) or by the increase in absorbance caused by the reduction of DTNB [5,5'-dithiobis(2-nitrobenzoic acid)] at 412 nm (Colorimetric assay).

Glutathione reductase is a homodimeric enzyme with a molecular mass ranging from 100 kDa for the human erythrocyte enzyme to 118 kDa for the yeast enzyme. It contains one FAD moiety per subunit and shows a K_M of 4–9 μM for NADPH and 55–65 μM for GSSG. The enzyme is inhibited by divalent metal ions (Zn^{2+} or Cd^{2+}), but this can be prevented by the addition of EDTA to the extraction buffer. The activity of this enzyme will be depressed by ammonium sulfate at concentrations greater than 60 mM, but 0.6 M urea, 1% TRITON® X-100, or 100 mM KCl have very little effect on the activity.

Components

The kit is sufficient for 100 assays.

- Glutathione Reductase Assay Buffer 125 ml
100 mM potassium phosphate buffer, pH 7.5, with 1 mM EDTA (Catalogue Number G8789)
- Glutathione Reductase Dilution Buffer 100 ml
100 mM potassium phosphate buffer, pH 7.5, with 1 mM EDTA and 1 mg/ml bovine serum albumin (Catalogue Number G0790)
- Glutathione Reductase Positive Control 1 vial
Lyophilized powder containing yeast glutathione reductase, potassium phosphate buffer, pH 7.5, with EDTA and trehalose as a stabilizer (Catalogue Number G0665)
- β -Nicotinamide Adenine Dinucleotide Phosphate, Reduced (NADPH) 25 mg
(Catalogue Number MUS84)
- Glutathione, Oxidized, Disodium Salt 100 mg
(Catalogue Number G4626)
- 5,5'-Dithiobis(2-nitrobenzoic acid) 50 mg
(Catalogue Number D8130)

Equipment Required but Not Provided

UV/Vis spectrophotometer with thermostated cuvette holder and a kinetic program. Use quartz cuvettes for the UV assay.

Precautions and Disclaimer

For R&D use only. Not for drug, household, or other uses. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.

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Storage/Stability

Store all kit components at 2–8 °C. The components in this kit are stable for 24 months when unopened.

Note: All the solutions supplied in this kit have been filtered through a 0.2 µm filter and the bottles are aseptically filled. For long term stability of the solutions after opening, handle the solutions in an aseptic manner. No preservative has been added to these solutions

Preparation Instructions

Prepare the solutions in the volume required for the number of assays to be performed, according to the detection method to be used.

Glutathione Reductase Positive Control Solution - Reconstitute the vial with 1 ml of water to obtain a glutathione reductase activity of >1 unit per ml in a solution containing 100 mM potassium phosphate buffer, pH 7.5, with 1 mM EDTA and 38 mg/ml trehalose. For long term storage, divide the solution into aliquots and freeze at –20 °C. The solution will be stable for at least 6 months at –20 °C.

2 mM NADPH Solution - Dissolve a portion of the β-Nicotinamide adenine dinucleotide phosphate, reduced (NADPH) at 1.85 mg/ml in Assay Buffer to prepare a working solution of 2 mM. Store at 4 °C. Prepare the NADPH solution fresh every day.

2 mM Oxidized Glutathione Solution - Dissolve the Glutathione, Oxidized, Disodium salt (GSSG) at 1.42 mg/ml in Assay Buffer to prepare a working solution of 2 mM. Store at 25 °C while performing the test. The solution may be kept up to 7 days at 2–8 °C for temporary storage.

3 mM DNTB Solution - Dissolve the 5,5'-Dithiobis(2-nitrobenzoic acid) (DNTB) at 1.19 mg/ml in Assay Buffer to prepare a working solution of 3 mM. This solution is very unstable; prepare the solution fresh every 4 hours and store at 4 °C.

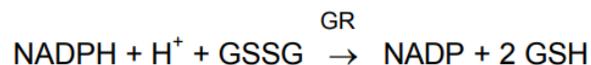
Sample Preparation

Dilute the samples to be assayed in Dilution Buffer as needed immediately before assaying. The concentration dependent enzymatic reaction is linear from 0.003–0.03 units per ml of reaction mixture for the colorimetric assay and from 0.003–0.012 units per ml of reaction mixture for the UV assay.

Procedure

This assay is based on the reduction of glutathione (GSSG) by NADPH in the presence of glutathione reductase. In addition, 5,5'-dithiobis(2-nitrobenzoic

acid) [DTNB] reacts with the reduced glutathione (GSH) formed:



The reduced glutathione can then spontaneously react with DTNB:



TNB = 5-thio(2-nitrobenzoic acid)

The first reaction is measured by the decrease in absorbance at 340 nm using an extinction coefficient (ϵ^{mM}) of 6.22 for NADPH and the second reaction is measured by the increase in absorbance at 412 nm using an extinction coefficient (ϵ^{mM}) of 14.15 for TNB.

Unit definitions:

UV assay - One unit will cause the oxidation of 1.0 µmole of NADPH at 25 °C at pH 7.5.

Colorimetric assay - One unit will cause the reduction of 1.0 µmole of DTNB to TNB at 25 °C at pH 7.5.

Assay Reaction

1. Equilibrate the Assay Buffer and 2 mM Oxidized Glutathione Solution at 25 °C for at least 10 minutes before starting the assay.
2. Set up the kinetic program in the spectrophotometer. The following programs are recommended:
 - a. UV assay:
Wavelength: 340 nm
Initial delay: 10 seconds
Interval: 10 seconds
Number of readings: 11
 - b. Colorimetric assay:
Wavelength: 412 nm
Initial delay: 60 seconds
Interval: 10 seconds
Number of readings: 11
3. Zero the spectrophotometer with a cuvette filled with water.
4. Place the following solution volumes in a 1 ml quartz cuvette in the order shown in Table 1.

Table 1.
Reaction Volumes

Solution	UV assay	Colorimetric assay
2 mM Oxidized Glutathione	500 μ l	500 μ l
Assay Buffer	350-450 μ l	50-150 μ l
Sample*	0-100 μ l	0-100 μ l
3 mM DTNB	----	250 μ l
2 mM NADPH	50 μ l	50 μ l
Total volume	1.00 ml	1.00 ml

*For both (UV or Colorimetric) assays perform a positive control by adding 10 to 20 μ l of the Glutathione Reductase Positive Control Solution per control reaction.

- The reaction is started by the addition of the NADPH solution. Mix by inversion, place the cuvette in the spectrophotometer, and start the kinetic program.
- Run the reaction with Assay Buffer instead of enzyme sample solution as a blank.
- Calculate the amount of enzyme in the sample.

Results

Calculation

- The spectrophotometer should record the ΔA /minute for the reaction automatically. If the absorbance is measured manually, calculate this value for the blank, positive controls, and all samples.
- Samples that give a large deviance from linearity should not be taken into account. This may happen with very dilute or very concentrated samples. The range of enzymatic activity that can be measured varies with the assay used. The concentration dependent enzymatic reaction is linear from 0.003–0.03 units per ml of reaction mixture for the colorimetric assay and from 0.003–0.012 units per ml of reaction mixture for the UV assay.
- The concentration of enzyme can be calculated using the formula:

$$\text{units/ml} = \frac{(\Delta A_{\text{sample}} - \Delta A_{\text{blank}}) \times (\text{dilution factor})}{\epsilon^{\text{mM}} \times (\text{volume of sample in ml})}$$

$$\text{For NADPH } \epsilon^{\text{mM}} = 6.22 \text{ mM}^{-1}\text{cm}^{-1}$$

$$\text{For TNB}^6 \epsilon^{\text{mM}} = 14.15 \text{ mM}^{-1}\text{cm}^{-1}$$

Applications

This product has been tested on a rabbit reticulocyte lysate and on *Saccharomyces cerevisiae* (yeast) cell extract, and was found to be suitable for the determination of glutathione reductase activity with these crude extracts. Tissues containing high levels of hemoglobin should be perfused with an isotonic saline solution containing 1 mM PMSF before homogenization to lower the endogenous hemoglobin levels, enabling the determination of GR activity with the colorimetric as well as the UV assay. Samples may be stored frozen at -70 °C. A typical rabbit reticulocyte lysate contained ~ 0.25 unit/mg of protein by the UV method only and a *S. cerevisiae* cell extract had ~ 0.32 units/mg of protein by both methods.

References

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