

GLUTATHIONE REDUCTASE

UV Method
For Research Use Only

50 Tests

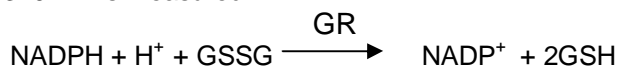
INTRODUCTION :

Glutathione reductase (GR) catalyzes the reduction of oxidized glutathione (GSSG) to reduced glutathione (GSH). Glutathione reductase is essential for the glutathione redox cycle that maintains adequate levels of reduced cellular GSH. GSH serves as an antioxidant, reacting with free radicals and organic peroxides, in amino acid transport, and as a substrate for the glutathione peroxidases and glutathione S-transferases in the detoxification of organic peroxide and metabolism of xenobiotics, respectively .

Assay of Glutathione Reductase has been used in the detection of hepatic and malignant disease, nutrition (assessment of riboflavin status) and detection of genetically determined deficiency states . Care must be taken to ensure that apparent enzyme deficiency states are not due to riboflavin depletion .

PRINCIPLE :

Glutathione reductase catalyses the reduction of glutathione (GSSG) in the presence of NADPH, which is oxidized to NADPH⁺ . The decrease in absorbance at 340 nm is measured .



REFERENCE RANGE :

Plasma / Serum 40 – 80 U / L

It is recommended that each laboratory should assign its own normal range .

SAMPLE:

Serum, plasma, erythrocytes, Cell lysate and tissue.

REAGENTS :

1.	Buffer Potassium phosphate pH 7.5 EDTA	100 mmol/L 1 mmol / L
2.	Substrate GSSG	50 mmol / L
3.	NADPH	2 mmol / L

PREPARATION OF SOLUTIONS :

1. Buffer (R1)
Contents ready for use. Stable up to the expiry date when stored at +2 to +8 °C
2. Substrate (R2)
Reconstitute the contents of substrate with 5 ml of buffer R1 . Stable for 2 months when stored at -20°C
3. NADPH (R3)
Reconstitute the content of NADPH with 5 ml of redistilled H₂O. Stable for 24 hrs when stored at -4 °C

PROCEDURE:

Pipette into cuvette one cm light path	
Sample	0.050 ml
Buffer (R1)	1.00 ml
GSSG (R2)	0.10 ml
NADPH (R3)	0.10 ml

Mix, assay is carried out at 25 °C, read against air the initial absorbance at 340 nm, start timer simultaneously . Read again after one min. over a period of 5 min, obtain the change in absorbance per min. (ΔA_{340} nm / min .) Linearity up to 0.25 ΔA_{340} nm / min . the detection limit is 10 U / L .

CALCULATION :

Glutathione Reductase Activity

$$(\text{U} / \text{L}) = 4019 \times \Delta A_{340} \text{ nm} / \text{min}$$

SAMPLE PREPARATION:

Tissue Homogenate

1. Prior to dissection, perfuse tissue with a PBS (phosphate buffered saline) solution, pH 7.4 . containing 0.16 mg / ml heparin to remove any red blood cells and clots.
2. Homogenize the tissue in 5 – 10 ml cold buffer (i , e , 50 mM potassium phosphate, pH 7.5. 1 mM EDTA) per gram tissue.
3. Centrifuge at 4,000 rpm for 20 minutes at +4 °C .
4. Remove the supernatant for assay and store on ice. If not assaying on the same day , freeze the sample at - 80°C. The sample will be stable for at least one month.

Cell Lysate

1. Collect cells by centrifugation (i , e , 1,000 – 2,000 rpm for 10 min. at +4 °C). For adherent cells, do not harvest using proteolytic enzymes; rather use a rubber policeman .
2. Homogenize cell pellet in cold buffer (i , e , 50 mM potassium phosphate, pH 7.5. 2 mM EDTA) .
3. Centrifuge at 4,000 rpm for 15 min. at +4 °C .
4. Remove the supernatant for assay and store on ice. If not assaying on the same day , freeze the sample at - 80°C. The sample will be stable for at least one month.

Plasma and Erythrocyte Lysate

1. Collect blood using an anticoagulant such as heparin, citrate , or EDTA .
2. Centerifuge the blood at 4,000 rpm for 10 minutes at +4 °C . Pipet off top yellow plasma layer without disturbing the white buffy layer. Store plasma on ice until assaying or freeze at -80 °C. The plasma sample will be stable for at least one month .
3. Remove the white buffy layer and discard .
4. Lyse the erythrocytes (red blood cells) in 4 times its volume of ice – cold HPLC grade water .
5. Centrifuge at 4,000 rpm for 15 minutes at +4 °C .
6. Collect the supernatant (erythrocyte lysate) for assaying and store on ice. If not assaying on the same day , freeze at - 80°C. The sample will be stable for at least one month.

REFERENCE :

Goldberg D.M. & Spooner RJ (1983) in Methods of Enzymatic Analysis (Bergmeyer, H.V. Ed.) 3rd edn. Vol 3, pp 258 – 265, Verlog Chemie, Deerfield beach, FI .

GLUTATHIONE REDUCTASE

UV Method	
R1 +2 to +8°C	50
R2 and R3 at -20 °C	Tests
CAT. NO.	GR 25 23

REAGENTS

R1	Buffer	50 ml
R2	Substrate	1 vial
R3	NADPH	1 vial

CONTACTS

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