RANDOX

GLUTATHIONE REDUCTASE

Manual

INTENDED USE.
For the quantitative *in vitro* determination of Glutathione Reductase in serum , plasma and erythrocytes. This product is suitable for Manual use.

Cat No.

GR 2368	1. Buffer	1 x 70 ml
5 x 5 ml	2. Substrate	5 x 5 ml
	3. NADPH	5 x 3 ml

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

Assay Principle[1]
Glutathione reductase catalyses the reduction of glutathione (GSSG) in the presence of NADPH, which is oxidised to $NADP^+$. The decrease in absorbance at 340 nm is measured.

$$NADPH + H^+ + GSSG \xrightarrow{GR} NADP^+ + 2\ GSH$$

(GSH = Reduced Glutathione)

SAMPLE PREPARATION
Serum, Plasma or Erythrocytes.
Erythrocyte preparation.
Centrifuge 0.5 ml of whole blood for 5 min at 2000 rpm. Remove the plasma and buffy coat, taking care not to remove too many erythrocytes, thereby risking non-representative cell sampling. Wash the erythrocytes three times by resuspending in 0.9% NaCl, centrifuging for 5 min at 2000 rpm after each wash.

Lyse the cells by resuspending in cold redistilled H_20, back up to 0.5 ml. Leave for 10 min at +2 - +8°C. Centrifuge lysate for 5 min at 2000 rpm to remove stroma. Dilute
100 µl of lysate with 1.9 ml of 0.9% NaCl solution for assay.

REAGENT COMPOSITION

Contents	Concentrations in the test
1. Buffer	
Potassium phosphate	250 mmol/l pH 7.3
EDTA	0.5 mmol/l
2. Substrate	
GSSG	2.2 mmol/l
3. NADPH	0.17 mmol/l

SAFETY PRECAUTIONS AND WARNINGS
For *in vitro* diagnostic use only. Do not pipette by mouth. Exercise the normal precautions required for handling laboratory reagents.
Health and Safety Data Sheets are available on request.

The reagents must be used only for the purpose intended by suitably qualified laboratory personnel, under appropriate laboratory conditions.

STABILITY AND PREPARATION OF REAGENTS
1. **Buffer**
   Contents ready for use. Stable up to the expiry date when stored at +2 to +8°C.

2. **Substrate**
   Reconstitute the contents of one vial of substrate 2 with **5 ml** of buffer 1. Stable for 2 days when stored at +2 to +8°C.

3. **NADPH**
   Reconstitute one vial of NADPH 3 with **3 ml** of redistilled H_20. Stable for 2 days when stored at +2 to +8°C.

MATERIALS PROVIDED
Buffer
Substrate
NADPH

MATERIALS REQUIRED BUT NOT PROVIDED
Randox Glutathione Reductase Control Sera GR 2608.

PROCEDURE FOR SERUM/PLASMA

Wavelength:	340 nm
Cuvette:	1 cm light path
Temperature:	37°C
Measurement:	against air
Pipette into cuvette	
Sample	40 µl
Substrate	1000 µl
Mix well.	
NADPH	200 µl

Mix, and start timer simultaneously. Read initial absorbance after 1 minute. Read again after 2, 3, 4 and 5 minutes.

CALCULATION
Glutathione Reductase activity may be calculated from the following formula:

U/l = 4983 x ΔA 340 nm/min

PROCEDURE FOR ERYTHROCYTES

Wavelength:	340 nm
Cuvette:	1 cm light path
Temperature:	37ºC.
Measurement:	against air
Pipette into cuvette	
Diluted Lysate	40 µl
Substrate	1000 µl
Mix well	
NADPH	200 µl

Mix, and start timer simultaneously. Read initial absorbance after 1 minute. Read again after 2, 3, 4 and 5 minutes.

CALCULATION

The Glutathione Reductase activity may be calculated from the following formula.

a) U/l whole blood = 4983 x ΔA 340 nm/min x Dilution Factor (20)
b) Converting to u/g Haemoglobin

example

A sample is found to contain a haemoglobin level of 16 g/dl or 160 g/l.
Glutathione Reductase value: 1488 U/l.

Therefore the sample value = $\frac{1488}{160} = 9.3$ u/gHb

QUALITY CONTROL

Randox Glutathione Reductase Control Sera is recommended for daily quality control. The control should be assayed at least once a day. Values obtained should fall within a specified range. If these values fall outside the range and repetition excludes error the following steps should be taken:

1. Check instrument settings and light source.
2. Check cleanliness of all equipment in use.
3. Check water, contaminants ie bacterial growth may contribute to inaccurate results.
4. Check reaction temperature.
5. Check expiry date of kit and contents.
6. Contact Randox Laboratories Customer Technical Support, Northern Ireland (028) 94422413.

SPECIFICITY

Glutathione Reductase is highly specific for Glutathione(GSSG).

REFERENCE RANGE[(2)]

Plasma/Serum	33 - 73 U/l
Erythrocytes	4.7 -13.2 U/gHb

It is recommended that each laboratory establish its own reference range to reflect the age, sex, diet and geographical location of the population.

LINEARITY

If the absorbance change/minute exceeds 0.06 at 340 nm, dilute 0.1 ml of sample with 0.9 ml of buffer 1. Multiply result by 10.

SENSITIVITY

A value of 10 U/l should be considered as the detection limit.

REFERENCE

1. Goldberg D.M. & Spooner RJ (1983) in Methods of Enzymatic Analysis (Bergmeyen, H.V. Ed.) 3rd edn. vol 3, pp 258-265, Verlog Chemie, Deerfield Beach, Fl.
2. Melissinos, K.G.,Delidov, A.Z., Varsov, A.G., Begietti, S.S., Drivas, G.J., Nephron, **28**: 76-79, (1981).

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RANDOX Laboratories Ltd., Ardmore, Diamond Road, Crumlin, Co. Antrim, United Kingdom, BT29 4QY
Tel:CRUMLIN (028)94422413 Fax.No.INT.44 (028)94452912 UK (028)94452912
Email: applications@randox.com Website: www.randox.com