RANSEL

Glutathione Peroxidase
Manual

INTENDED USE

For the quantitative *in vitro* determination of Glutathione Peroxidase in whole blood. This product is suitable for Manual use.

Cat. No.		
RS 504	1. Reagent	8 x 6.5 ml
8 x 6.5 ml	2. Buffer	1 x 70 ml
8 x 6 t Semi micro	3. Cumene Hydroperoxide	1 x 1 ml
8 x 2 t Macro	4. Diluting Agent	2 x 200 ml
RS 505	1. Reagent	8 x 10 ml
8 x 10 ml	2. Buffer	1 x 100 ml
8 x 10 t Semi micro	3. Cumene Hydroperoxide	1 x 1 ml
8 x 4 t Macro	4. Diluting Agent	2 x 200 ml
RS 506	1. Reagent	8 x 30 ml
8 x 30 ml	2. Buffer	1 x 250 ml
8 x 30 t Semi micro	3. Cumene Hydroperoxide	1 x 1 ml
8 x 12 t Macro	4. Diluting Agent	4 x 200 ml

Supplementary Pack:

MS 181	Drabkin's Reagent	6 x 500 ml

UV METHOD [1]

This method is based on that of Paglia and Valentine. Glutathione Peroxidase (GPX) catalyses the oxidation of Glutathione (GSH) by Cumene Hydroperoxide. In the presence of Glutathione Reductase (GR) and NADPH the oxidised Glutathione (GSSG) is immediately converted to the reduced form with a concomitant oxidation of NADPH to $NADP^+$. The decrease in absorbance at 340 nm is measured.

REACTION PRINCIPLE

$$2GSH + ROOH \xrightarrow{GPX} ROH + GSSG + H_2O$$

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

SAMPLE PREPARATION

Use heparinized whole blood.
For sheep and goats: dilute 0.05 ml + 3 ml diluting agent.
For cattle, horses and other species: dilute 0.05 ml + 2 ml diluting agent.
For human samples: see NOTE.

REAGENT COMPOSITION

Contents	Concentration in the Test
1. Reagent	
Glutathione	4 mmol/l
Glutathione Reductase	≥ 0.5 U/l
NADPH	0.34 mmol/l
2. Buffer	
Phosphate Buffer	0.05 mol/l; pH 7.2
EDTA	4.3 mmol/l
3. Cumene Hydroperoxide	0.18 mmol/l
4. Diluting Agent	

SUPPLEMENTARY REAGENT COMPOSITION

Contents	Initial Concentration of Solutions
Drabkin's Reagent	
Potassium Phosphate	104 mmol/l
Potassium Ferricyanide	60.8 mmol/l
Potassium Cyanide	78.8mmol/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.

Solution 2 contains Sodium Azide. Avoid ingestion or contact with skin or mucous membranes. In case of skin contact, flush affected area with copious amounts of water. In case of contact with eyes, or if ingested, seek immediate medical attention.

Sodium Azide reacts with lead and copper plumbing, to form potentially explosive azides. When disposing of such reagents flush with large volumes of water to prevent azide build up. Exposed metal surfaces should be cleaned with 10% sodium hydroxide.

Solution 3 is harmful if swallowed or inhaled. It causes irritation and is combustible.

Solution 3 is Cumene Hydroperoxide which is caustic.

Drabkin's Solution contains cyanide and may be fatal if swallowed.

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. **Reagent**
   Reconstitute one vial of Reagent 1 with the appropriate volume of Buffer 2:

6.5 ml	for the	**8 x 6.5 ml**	kit (RS 504)
10 ml	for the	**8 x 10 ml**	kit (RS 505)
30 ml	for the	**8 x 30 ml**	kit (RS 506)

   Stable for 48 hours at +2 to +8°C or 8 hours at +15 to +25°C.

2. **Buffer**
   Contents ready for use. Stable up to expiry date when stored at +2 to +8°C.

3. **Cumene Hydroperoxide**
   Dilute **10 µl** with **10 ml** of redistilled water and mix thoroughly by shaking vigorously as the cumene is difficult to dissolve. Prepare fresh daily. Concentrate stable up to the expiry date when stored at +2 to +8°C. A pipette with a positive displacement action, and using glass capillaries, should be used to measure the cumene hydroperoxide volume.

4. **Diluting Agent**
   Reconstitute the contents of one vial of Diluting Agent 4 with **200 ml** of redistilled water. Stable for 4 weeks when stored at +2 to +8°C or 3 days at +15 to +25°C.

MATERIALS PROVIDED

Reagent
Buffer
Cumene Hydroperoxide
Diluting Reagent

MATERIALS REQUIRED BUT NOT PROVIDED

Ransel Control (Cat. No. SC 692)
Redistilled water
Drabkin's Reagent (Cat. No. MS 181)
Ransel Diluent (Cat No. RS 2318)

NOTE [2,3]
When using human heparinized whole blood, it is recommended that Drabkin's reagent is used for dilution. This is due to the presence of peroxidases in human blood which may give falsely elevated results, and the addition of cyanide serves to inhibit this positive interference. However, dilution of the blood with diluting agent (solution 4) is necessary prior to addition of Drabkin's to convert the glutathione to the reduced form. This is because, in the oxidized form, cyanide will quickly lead to inactivation.

The following method is recommended using double strength Drabkin's:

Preparation: Dilute the contents of one vial of Drabkin's reagent with 480 ml of redistilled water. Store protected from light. Stable for 6 months or to expiry date, whichever is the shortest, when stored at +15 to +25°C.

Dilute 0.05 ml heparinized whole blood with 1 ml diluting agent; incubate for 5 minutes and add 1 ml of double strength Drabkin's reagent. Mix well and assay in the normal manner. It is recommended that the samples are assayed within 20 minutes of adding the Drabkin's reagent.

PROCEDURE

Wavelength:	340 nm
Cuvette:	1 cm light path
Temperature:	37°C
Measurement:	against air

Pipette into cuvette:

	Macro		Semi-Micro	
	Diluted Sample	Reagent Blank	Diluted Sample	Reagent Blank
Diluted Sample	0.05 ml	---	0.02 ml	---
Distilled H_2O	---	0.05 ml	---	0.02 ml
Reagent	2.50 ml	2.50 ml	1.00 ml	1.00 ml
Cumene	0.10 ml	0.10 ml	0.04 ml	0.04 ml

Mix, read initial absorbance of sample and reagent blank after one minute and start timer simultaneously. Read again after 1 and 2 minutes. Subtract reagent blank value from that of the sample.

CALCULATION
Glutathione Peroxidase Concentration may be calculated from the following formula:

U/l of Haemolysate = 8412 x ΔA 340 nm / minute

(See technical brief for example).

QUALITY CONTROL
A Ransel Control 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 i.e. 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.

REFERENCE RANGE
27.5 - 73.6 U/g Hb
4171 - 10881 U/l

The range was measured in a European working population. It is recommended that each laboratory should assign its own normal range.

LINEARITY
If the absorbance change per minute exceeds 0.1 at 340 nm, dilute sample accordingly with diluting agent, and multiply the result by the dilution factor.

REFERENCES
1. Paglia, D.E. and Valentine, W.N., J. Lab. Clin. Med., 1967; **70**: 158.
2. Kraus, R.J. & Ganther, H. E. Biochem. & Biophys. Res. Comm 1980; **96**: 1116.
3. Prohaska, J.R., Oh, S.H., Hoekstra, W.G. & Ganther, H.E. Biochem. & Biophys. Res. Comm. 1977; **74**: 64.

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