RANSEL

Glutathione Peroxidase
MANUAL
RX MONZA

INTENDED USE

For the quantitative *in vitro* determination of Glutathione Peroxidase in whole blood. This product is suitable for manual use and on the RX **monza** analyser.

Cat. No.			
RS 504	R1a.	Reagent	8 x 6.5 mL
8 x 6.5 mL	R1b.	Buffer	1 x 70 mL
	R2.	Cumene Hydroperoxide	1 x 1 mL
	R3.	Diluting Agent	2 x 200 mL
GTIN:	05055273205305		
RS 505	R1a.	Reagent	8 x 10 mL
8 x 10 mL	R1b.	Buffer	1x 100 mL
	R2.	Cumene Hydroperoxide	1 x 1 mL
	R3.	Diluting Agent	2 x 200 mL
GTIN:	05055273205312		

Supplementary Pack:

HG 1539	Haemoglobin Reagent	5 x 100 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.
Additional dilutions may be required for samples above the assay range.

For human samples: see NOTE.

REAGENT COMPOSITION

Contents		Concentration in the Test
R1a.	**Reagent**	
	Glutathione	4 mmol/L
	Glutathione Reductase	≥ 0.5 U/L
	NADPH	0.34 mmol/L
R1b.	**Buffer**	
	Phosphate Buffer	0.05 mol/L; pH 7.2
	EDTA	4.3 mmol/L
R2.	**Cumene Hydroperoxide**	0.18 mmol/L
R3.	**Diluting Agent**	

SUPPLEMENTARY REAGENT COMPOSITION

Contents	Initial Concentration of Solutions
Haemoglobin Reagent	
Potassium Phosphate	10.3 mmol/L
Potassium Ferricyanide	6.08 mmol/L
Potassium Cyanide	7.68 mmol/L
Surfactant	0.1% v/v

SAFETY PRECAUTIONS AND WARNINGS

For *in vitro* diagnostic use only. Do not pipette by mouth. Exercise the normal precautions required for handling laboratory reagents.

This kit contains components classified as hazardous in accordance with the Regulation (EC) No.1272/2008:
R2. Cumene Hydroperoxide

Signal Word(s) Danger

Hazard statement(s)

H242	Heating may cause a fire.
H302	Harmful if swallowed.
H304	May be fatal if swallowed and enters airways.
H312	Harmful in contact with skin.
H314	Causes severe skin burns and eye damage.
H332	Harmful if inhaled.
H335	May cause respiratory irritation.
H373	May cause damage to organs through prolonged or repeated exposure.
H411	Toxic to aquatic life with long lasting effects.

Precautionary statement(s)

P261	Avoid breathing dust/ fume/ gas/ mist/ vapours/ spray.
P273	Avoid release to the environment.
P280	Wear protective gloves/protective clothing/eye protection/face protection.
P301+P310	IF SWALLOWED: IF SWALLOWED: Immediately call a POISON CENTER/doctor.
P301+P330+P331	IF SWALLOWED: rinse mouth. Do NOT induce vomiting.
P305+P351+P338	IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.

Disposal of all waste material should be in accordance with local guidelines.
Safety data sheet available on request.

Solution R1b and R3 contain 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.

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

STABILITY AND PREPARATION OF REAGENTS

R1a. Reagent

Reconstitute one vial of Reagent R1a with the appropriate volume of Buffer R1b:

6.5 mL for the **8 x 6.5 mL** kit (RS 504)
10 mL for the **8 x 10 mL** kit (RS 505)

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

R1b. Buffer

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

R2. Cumene Hydroperoxide

Dilute **10 µL** R2 with **10 mL** of saline 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.

R3. Diluting Agent

Reconstitute the contents of one vial of Diluting Agent R3 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.

Please note that reagent preparation steps may change if Ransel is used on an automated system. A range of instrument-specific applications for Ransel are available from applications@randox.com.

MATERIALS PROVIDED

Reagent
Buffer
Cumene Hydroperoxide
Diluting Agent

MATERIALS REQUIRED BUT NOT PROVIDED

Ransel Control (Cat. No. SC 692)
Redistilled water
Haemoglobin Reagent (Cat. No. HG 1539)
Ransel Diluting Agent (Cat No. RS 2318)
Ransel Calibrator (Cat. No. SC 10154)

NOTE [2, 3]

When using human heparinized whole blood, it is recommended that Haemoglobin 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 (R3) is necessary prior to addition of Haemoglobin reagent 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 Haemoglobin Reagent:

Preparation: Dilute 1 volume of the Haemoglobin reagent with 4 volumes 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 (R3); incubate for 5 minutes at +25°C and add 1 mL of Haemoglobin reagent. Mix well and assay in the normal manner. It is recommended that the samples are assayed within 20 minutes of adding the Haemoglobin reagent.

PROCEDURE

Select GPx in the Run Test screen and carry out a water blank as instructed.

Pipette into a test tube:

Sample	10 µL
Reagent R1	500 µL
Cumene R2	20 µL

Mix and aspirate into the Rx Monza.

CALIBRATION FOR RX MONZA

Recommended daily or as indicated by quality control procedures, using Randox Ransel Calibrator.

FOR MANUAL USE

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 Calibrator	0.05 mL	---	0.02 mL	---
Diluted Sample	0.05 mL	---	0.02 mL	---
Distilled H_2O	---	0.05 mL	---	0.02 mL
Reagent R1	2.50 mL	2.50 mL	1.00 mL	1.00 mL
Cumene R2	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.

MANUAL CALCULATION

Glutathione Peroxidase Concentration may be calculated from the following formula:

U/L of Haemolysate = *8412 x –A 340 nm / minute

*Factor should be determined using the Ransel Calibrator for each new batch of reagent
Factor = ((Cal ABS - Blank ABS)/Cal Target)

(See technical brief for example of calculation).

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 Technical Services, Northern Ireland +44 (0) 28 94451070.

INTERFERENCE

Physiological changes in serum or plasma analyte concentrations can be caused by a number of substances. Comprehensive discussion of possible interfering substances, their serum or plasma concentrations, and their possible physiological involvements is beyond the scope of this document. The listed reference contains specific details on known potential interfering substances [4]. The user must remain vigilant to the possible effect on results of unknown interferences from medications or endogenous substances. All patient results must be evaluated in light of the total clinical status of the patient.

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.

SPECIFIC PERFORMANCE CHARACTERISTICS

The following performance data were obtained using an Rx Monza analyser in flow cell mode at +37°C with an aspiration volume of 450 µL.

LINEARITY

The method is linear up to a concentration of 925 U/L. Dilute samples with concentration greater than this with diluting agent and multiply the result by the dilution factor.

SENSITIVITY

The minimum detectable concentration of Gluthathione Peroxidase with an acceptable level of precision was determined as 75 U/L.

PRECISION

Within run precision

	Level 1	Level 2
Mean (U/L)	240	456
SD	11.7	14.6
CV (%)	4.86	3.20
n	20	20

Total precision

	Level 1	Level 2
Mean (U/L)	240	456
SD	17.5	19.9
CV (%)	7.30	4.37
n	20	20

CORRELATION

The Randox method (Y) was compared to method (X). Forty patient samples with values spanning the range
102 - 650 U/L were tested. Linear regression analysis gave the following equation:

Y= 1.0155 X +8.637

with a correlation coefficient of r = 0.9829

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.
4. Young DS. Effects of Drugs on Clinical Laboratory Tests. 5th ed. Washington, DC: AACC Press; 2000.

The presence of a vertical bar in the margin indicates a Technical update from the previous revision.

EC REP Randox Teoranta, Meenmore, Dungloe, Donegal, F94 TV06, Ireland

Revised 05 Mar 24 ld

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