RANDOX

CE

GLUCOSE-6-PHOSPHATE DEHYDROGENASE (G-6-PDH)

MANUAL
RX MONZA

INTENDED USE

For the quantitative *in vitro* determination of Glucose-6-Phosphate Dehydrogenase in erythrocytes. This product is suitable for manual use and on the RX **monza** analyser.

Cat. No.

PD 410	R1. Buffer	1 x 100 ml
100 ml	R2. NADP	1 x 2 ml
	R3. Substrate	1 x 2 ml
	R4. Digitonin	1 x 20 ml

UV METHOD

PRINCIPLE[1]

The enzyme activity is determined by measurement of the rate of absorbance change at 340 nm due to the reduction of $NADP^+$.

$$G\text{-}6\text{-}P + NADP^+ \xrightarrow{G\text{-}6\text{-}PDH} gluconate\text{-}6\text{-}P + NADPH + H^+$$

SAMPLE

Erythrocytes

PREPARATION OF SAMPLE

Wash 0.2 ml of blood with 2 ml aliquots of 0.9% NaCl solution. Centrifuge after each wash for 10 min at around 3000 rpm. Repeat 3 times. Suspend the washed centrifuged erythrocytes in 0.5 ml of solution 4 and let stand for 15 min at +4°C and then centrifuge again. Use the supernatant in the assay within 2 hours.

REAGENT COMPOSITION

Contents	Concentrations in the Test
R1. Buffer	
Triethanolamine Buffer	31.7 mmol/l, pH 7.6
EDTA	3.2 mmol/l
R2. NADP	0.34 mmol/l
R3. Substrate	0.58 mmol/l
R4. Digitonin	

SAFETY PRECAUTIONS AND WARNINGS

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

Solutions R1, R2, R3 and R4 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.

Health and Safety Data Sheets are available on request.

Please dispose of all Biological and Chemical materials according to local guidelines.

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

STABILITY AND PREPARATION OF REAGENTS

R1. Buffer
Contents ready for use. Stable up to the expiry date when stored at +2 to +8°C.

R2. NADP
Reconstitute the contents of bottle R2 with **2 ml** of redistilled water. Stable for 4 weeks at +2 to +8°C.

R3. Substrate
Reconstitute the contents of bottle R3 with **2 ml** of redistilled water. Stable for 4 weeks at +2 to +8°C.

R4. Digitonin
Contents ready for use. Stable up to the expiry date specified when stored at +2 to +8°C.

MATERIALS PROVIDED

Buffer
NADP
Substrate
Digitonin

MATERIALS REQUIRED BUT NOT PROVIDED

Randox G-6-PDH Controls:
Deficient (Cat. No. PD 2617)
Normal (Cat. No. PD 2618).
Redistilled Water

Additional Digitonin may be required when running the G-6-PDH assay on automated systems. This can be purchased from Sigma (Cat No. D-5628). We recommend mixing 20mg Digitonin in 100ml deionised water until dissolved. Digitonin should be prepared fresh, weekly and stored at +2 to +8°C.

PROCEDURE
Determine the number of erythrocytes/ml of blood.

CALIBRATION
Select Curve Type "K factor" in calibration screen.
When asked: "Do you want to run S0 Calibration?", please select NO
Enter value "0.0" into the Concentration field for S0
Save changes

1. RX MONZA
Select G6PD in the Run Test screen and carry out a water blank as instructed.

Pipette into a test tube:

	Sample
Haemolysate	7.5 µl
Reagent R1	500 µl
Reagent R2	15 µl

Mix, incubate for 5 min at +37°C; then add:

Reagent R3	7.5 µl

Mix well and aspirate into the RX **monza**.

2. MANUAL USE

Wavelength:	340 nm (Hg 334 nm or Hg 365 nm)
Cuvette:	1 cm light path
Temperature:	+37°C
Measurement:	against air

Pipette into test tube:

	Macro	Semi Micro
R1	3.00 ml	1.00 ml
R2	0.10 ml	0.03 ml
Haemolysate	0.05 ml	0.015 ml

Mix, incubate for 5 minutes at +37°C; then add:

	Macro	Semi Micro
R3	0.05 ml	0.015 ml

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

MANUAL CALCULATION
To calculate the G-6-PDH activity use the following formulae:

Macro
mU/erythrocytes per ml blood* = 30476 x ΔA 340 nm/min
mU/erythrocytes per ml blood* = 54857 x ΔA Hg 365 nm/min
mU/erythrocytes per ml blood* = 31068 x ΔA Hg 334 nm/min

Semi Micro
mU/erythrocytes per ml blood* = 33650 x ΔA 340 nm/min
mU/erythrocytes per ml blood* = 60571 x ΔA Hg 365 nm/min
mU/erythrocytes per ml blood* = 34304 x ΔA Hg 334 nm/min

Temperature Correction
Please note that the below temperature correction may be used for patient samples only.

When the temperature is +37°C, no temperature correction factor (TCF) is required. If results for patient samples are reported at a temperature other than +37°C, a TCF must be used.

Cuvette Temperature (°C)	TCF
+25	2.076
+30	1.515

To calculate G-6-PDH activity as mU/10⁹ erythrocytes
Divide the calculated activity (mU/erythrocytes per ml blood) with the RBC's count per ml.

eg. RBC count per ml $= 5.3 \times 10^9$

mU/erythrocytes per ml = 695

mU/10^9 erythrocytes $= \frac{695}{5.3} = 131$

To calculate G-6-PDH activity as mU/g haemoglobin
The following equation is used.

$$\text{G-6-PDH mU/gHb} = \frac{\text{mU.erthrocytes per ml} \times 100}{\text{Hb (g/dl)}}$$

100 = Factor to convert from ml to dl

Hb(g/dl) = Haemoglobin concentration determined for each specimen

eg. mU/erythrocytes per ml = 695

Hb(g/dl) = 15.0

$$\text{G-6-PDH mU/gHb} = \frac{695 \times 100}{15.0}$$

= 4633

QUALITY CONTROL
Randox G-6-PDH Controls, Deficient and Normal are recommended for quality control to monitor accuracy and precision. Two levels of controls 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 Services, Northern Ireland +44 (0) 28 9445 1070.

INTERFERENCE
Reticulocytes have higher G-6-PDH levels than mature red cells. Therefore, it is not recommended that this assay be performed after a severe hemolytic crisis, since G6-PDH levels may appear falsely elevated. Testing may be more helpful after the level of mature red cells have returned to normal.

NORMAL VALUES[3]

In erythrocytes: 245 - 299 mU/10^9 erythrocytes (+37°C).

6.97 - 20.5 U/g Hb (+37°C)

	Blood Haemoglobin (g/dl)
Adult Males	13 - 18
Adult Females	11 - 16
Newborns	14 - 23

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

SPECIFIC PERFORMANCE CHARACTERISTICS

The following performance data were obtained using an RX **monza** analyser in flow cell mode running at +37°C.

LINEARITY

This method is linear up to a concentration of 4303 U/l. Dilute samples with concentration greater than this, using 0.2 ml of haemolysate with 1.8 ml of 0.9% NaCl solution and repeat the assay. Multiply the result by 10.

SENSITIVITY

The minimum detectable concentration of G6PDH in erythrocytes with an acceptable level of precision was determined as 154 U/l.

PRECISION

Intra assay precision

	Level 1	Level 2
Mean (U/l)	784	1533
SD	33.2	71.3
CV (%)	4.24	4.65
n	20	20

Inter assay precision

	Level 1	Level 2
Mean (U/l)	784	1533
SD	50.5	78.3
CV (%)	6.45	5.11
n	20	20

CORRELATION

The Randox method on the Rx Monza (Y) was compared to the Rx Daytona (X) and the following linear regression equation was obtained:

$Y = 1.0069\ x + 47.644$
and a correlation coefficient of $r = 0.9903$

50 patient samples were analysed spanning the range 162 to 1200U/l.

REFERENCES

1. Kornberg, A. *et al*., Methods in Enzymology 1, Academic Press, New York, 1955; p.323.
2. Makarem, A., Clinical Chemistry-Principles and Techniques. 2nd Ed. R.F. Henry, D. C. Cannon, J.W. Winkelman, Editors. Harper and Row, Hagerstown [MD], 1974; 1128-1135.
3. Lohr GW, Waller HD: Glucose-6-Phosphate Dehydrogenase. Methods of Enzymatic Analysis, 3rd Edition - Varlag Chemie, Wehnheim: 1974; p. 636.

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