GLUCOSE-6-PHOSPHATE DEHYDROGENASE

UV Method **25 Tests**

PRINCIPLE :

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

$$G6P + NADP^+ \xrightarrow{G6PDH} \text{gluconate-6-P} + NADPH + H^+$$

SAMPLE :

Erythrocytes

Whole blood collected with EDTA, heparin or ACD (Acid-Citrate-Dextrose).
Red Cell G6PDH is stable in whole blood for 1 week at 2 – 8°C, but is unstable in Red Cell hemolysate. Freezing of blood is not recommended.

NORMAL VALUES (at 25°C) :

Erythrocytes: 118 – 144 $mU/10^9$ erythrocytes
4.5 – 13.5 U/gHb

	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.
Values for the newborns may range somewhat higher.

REAGENTS :

		Initial Concentration
1.	**Buffer** Triethanolamine Buffer EDTA	 50 mmol / L, pH 7.6 5 mmol / L
2.	**NADP**	5 mmol / L
3.	**Glucose-6-phosphate**	30 mmol / L
4.	**Digitonin**	

PREPARATION OF SOLUTIONS:

The Reagents have to be used properly , to avoid contamination

1. **Buffer**
   Contents ready for use. Stable up to the expiry date when stored at +2 to +8°C.
2. **NADP**
   Reconstitute the contents of Reagent 2 with 1.0 ml of redistilled water. Stable for 2 months at -20°C.
3. **Substrate**
   Reconstitute the contents of Reagent 3 with 0.5 ml of redistilled water. Stable for 4 months at -20°C.
4. **Digitonin**
   Contents ready for use. Stable up to the expiry date specified when stored at +2 to +8°C.

REFERENCE :

Kornberg, A. et al., Methods in Enzymology 1, Academic Press, New York, 1955; p. 323.

PROCEDURE:

1-THE ASSAY OF G-6-PDH IN ERYTHROCYTES

Before the assay of G6PDH it is necessary to determine these things:

a- The number of erythrocytes (RBC) per ml blood to express G-6-PDH activity as $U/10^9$ erythrocytes (RBC), or
b- The concentration of haemoglobin in g/dL, to express G-6-PDH activity as U/g Hb.

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 3000 rpm. Repeat 3 times. Suspend the washed centrifuged erythrocytes in 0.5 ml of Reagent 4 and let stand for 15 min. at +4°C and then centrifuge again. Use the supernatant in the assay within 2 hours.

Pipette into test tube:-	
	Sample
Reagent 1 (Buffer)	1.00 ml
Reagent 2 (NADP)	0.03 ml
Haemolysate	0.015 ml
Mix, incubate for 5 minutes at 25°C, then add:	
Reagent 3 (G-6-P)	0.015 ml
Mix, assay is carried out at 25°C. Read initial absorbance against air at 340 nm using cuvette 1 cm light path. Start timer simultaneously, measure the absorbance change at exactly 3 minutes after the initial reading and divide by 3 to obtain ΔA_{340} nm/minute.	

CALCULATION :

- To calculate the G-6-PDH activity in blood use the following formula:

mU/ erythrocytes per ml blood = 33650 x Δ A 340 nm/min

G-6-PDH activity is expressed as:
a- $mU/10^9$ erythrocytes or
b- U/g haemoglobin.

- To calculate G-6-PDH activity as $mU/10^9$ erythrocytes, divide the calculated activity (mU/erythrocytes per ml blood) with the RBC's count per ml.

eg. RBC count per ml = 5.3×10^9
mU/ erythrocytes per ml = 695
$mU/10^9$ erythrocytes $= \frac{695}{5.3} = 131$

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

$$\text{G-6-PDH U/gHb} = \frac{\text{mU/ erythrocytes per ml} \times 100}{\text{Hb (g/dl)} \times 1000}$$

100 = Factor to convert activity to 100 ml

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

1000 = Factor to convert mU to U

eg. mU/ erythrocytes per ml = 695
Hb (g/dl) = 15

$$\text{G-6-PDH U/gHb} = \frac{695 \times 100}{15 \times 1000} = 4.633$$

LINEARITY:

If the absorbance change per minute exceeds 0.06 at 340 nm. Dilute 0.2 ml of haemolysate with 1.8 ml of 0.9% NaCl solution and repeat the assay, Multiply the result by 10.

If the G-6-PDH activity is very low, measure the absorbance change at exactly 5 minutes after the initial reading and divide by 5 to obtain ΔA/minute.

BIODIAGNOSTIC
DIAGNOSTIC AND RESEARCH REAGENTS

GLUCOSE-6-PHOSPHATE DEHYDROGENASE

UV Method

R1 and R4 at +2 to +8ºC
R2 and R3 at -20ºC 25 Tests
In vitro diagnostic use

CAT. NO. PD 25 26

REAGENTS

R1	**Buffer**	**25 ml**
R2	**NADP**	**1.0 ml**
R3	**Substrate**	**0.5 ml**
R4	**Digitonin**	**13 ml**

Contacts

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