G6PDH LS
(Quantitative/Kinetic Method)

Intended Use
Reagent Kit for quantitative estimation of G6PDH in human blood on photometric systems..

Reagent Provided
1. G6PDH Buffer Reagent – Ready to Use.
2. G6PDH Substrate Reagent – Ready to Use
3. G6PDH Lysing Reagent – Ready to Use

Principle
Glucose-6-Phosphate Dehydrogenase (G6PDH) catalyzes the first step in thepentose[hosphate shunt, oxidizing glucose-6-phosphate (G-6-P) to 6-phosphogluconate (6-PG) and reducing NADP to NADPH:

$$\text{G-6-P} + \text{NADP}^{+} \xrightarrow{\text{G6PDH}} \text{6-PG} + \text{NADPH} + \text{H}$$

NADP is reduced by G6PD in the presence of G-6-P. The rate of formation of NADPH is proportional to the G6PDH activity and is measured as increase in absorbance at 340 nm. Production of a second molar equivalent of NADPH by Erythrocyte 6-phosphogluconate dehydrogenase (6-PGDH) is prevented by use of maleimide, and inhibitor of 6-PGDH.

Components in the Working Reagent

Component	Concentration
NADP	2 mM
Malemide	15 mM
Glucose-6-Phosphate	1.05mM
Magnesium Salt	0.01 mM
Sodium Azide	<0.1%
Buffer Stablizers	

Reagent Storage & Stability
All the reagents are ready to use and stable till the expiry date mentioned on the labels.

Sample/Specimen
Fresh whole blood sample collected in EDTA, Heparin or ACD.

Red Cell G6PDH in whole blood is reported to be stable for 7 Days at 2-8°C but unstable in hemolysates. Freezing of whole blood is not recommended.

The integrity of erythrocytes collected in ACD is preserved even after prolonged storage so that obtaining accurate red cell counts usually poses no problem. However, red cell counts on specimens collected in heparin become unreliable after about 2 days. Thus, for heparinzed samples, results are best reported in terms of hemoglobin concentration.

Test Procedure
Step 1: Since activity is reported in terms of grams hemoglobin or the number of red blood cells, the **Hemoglobon Concentration or Red Cell count** must be determined prior to performing the G6PDH assay. Estimate first either Hb Concentration or RBC Count of that particular sample whose G6PDH Activity is to be determined.

Step 2:Preparation of Hemolysate
Take into a clean glass tube:

Lysing Reagent	1 ml
Whole Blood	10 µl

Mix well and incubate for 10 min at RT. This is the Hemolysate to be used for testing.

Step 3: Pipette into clean glass test tube the following:

Substrate	0.5 ml
Buffer	0.5 ml
Hemolysate Prepared Above	0.5 ml

Mix well and after 60 seconds incubation, measure the change of optical density per 60 seconds during next 180 seconds against distilled water at 340 nm .

Calculation
Calculate the average change in absorbance per minute (ΔAbs/min)

$$\text{Activity of G6PDH (U/gHb)} = \Delta Abs\ X\ \frac{4839}{\text{Hb(gm/dl)}}$$

$$\text{Activity of G6PDH (U/}10^{12}\text{aB}^{13}\text{)} = \Delta Abs\ X\ \frac{48390}{\text{RBC Count in Million}}$$

System Parameters

Programme 1

Reaction Type	Kinetic
Wavelength	340 nm
Slope of Reaction	Increasing
Temperature Setting	37°C
Zero Setting With	Distilled Water
Delay Time	60 sec
Measuring/Read Time	60 sec
No. of Readings	4
Reagent Volume	0.5 ml (Buffer) + 0.5 ml (Substrate)
Sample Volume	0.5 ml (Hemolysate)
Factor	4839 (When Hemoglobin is Measured)
	48390 (When RBC Count is measured)
Linearity	Up to (22.5 U/g Hb) or (628 U/10^{12} RBC'S)

Programme 2: System Parameters: For the confirmation of samples that came deficient in the above Programme 1

If the G6PDH activity is very low, the absorbance change per minute will also be very low. In such cases adapt the following timings and system parameters.

Reaction Type	Kinetic
Wavelength	340 nm
Slope of Reaction	Increasing
Temperature Setting	37°C
Zero Setting With	Distilled Water
Delay Time	300 sec
Measuring/Read Time	60 sec
No. of Readings	6
Reagent Volume	0.5 ml (Buffer) + 0.5 ml (Substrate)
Sample Volume	0.5 ml (Hemolysate)
Factor	4839 (When Hemoglobin is Measured)
	48390 (When RBC Count is measured)
Linearity	Up to (22.5 U/g Hb) or (628 U/10^{12} RBC'S)

Normal Reference Values

G6PDH Activity

(U/gHb) : 6.4 to 20.0 at 37°C

(U/10 RBC's) : 202 to 558 at 37°C

It is recommended that each laboratory should establish its own normal range representing its patient population.

Deficient Reference Values

G6PDH Activity: (u/gHb):Less than 6.4 at 37°C.

G6PDH Activity: (U/10 RBC's) : Less Than 202 at 37°C

It is recommended that each laboratory should establish its own deficient values representing its patient population.

Linearity

The maximum G6PDH activity which may be measured by this procedure is approx (22.5 U/gHb) or (628 U/10^{12}RBC's). if the value is greater than the reported value repeat determination using 5 µl blood as sample and multiply results by 2.

Sensitivity

Minimum change in absorbance ΔA/min = 0.001. Assuming a hemoglobin concentration of 12.0g/dl and are cell count of 4.5x10^{12}/mm3, a G6PDH activity of 0.4U/gHb or 11U/10^{12} RBC may be detected.

Method Comparison

A comparison study between High-QG6PDH (y) and a commercially available test (x) gave the following results:

Y=0.97x+0.07; r-0.994

Notes

1. Since the G6PDH activity is reported in Hb Concentration or RBC Count, the same should be estimated before performing the G6PDH assay. It means that the pathologist should estimate Hb Concentration or RBC Count of that particular whole blood sample whose G6PDH activity is to be determined.
2. RBC's are well preserved when collected in ACD and such samples can give an accurate count.
3. Samples collected in Heparin may give unreliable counts after 2 days and in such cases the results are best reported in Hb concentration.
4. Copper and sulphate ions inhibit G6PDH Activity and the care should be destroyed during mild to moderate hemolytic phase. Since reticulocytes released to replace lost cell have high enzyme levels, falsely elevated results may occur if blood is tested immediately after a hemolytic episode.
5. Normally the activity contributed by WBC, Platelet or Serum very small. Increase of severe anemia, leukocytosis or very low G6PDH levels. The use of a sample after removing the buffy coat is recommended.

References

1. Burtis, C.A., Ashwood, E.R., Tietz Textbook of Clinical Chemistry, W.B. Saunders, Philadelphia, p.p. 1645-1650, 1999.
2. Kornberg,A., Horecker, B.L.: Glucose-6-Phosphate Dehydrogenase.IN Methods in Enzymology. S.P. Colowick, N.O. Kaplan, Editors, Vol.I, Academic Press. New York, p323,1955.

Symbols

IVD In Vitro Diagnostics

Caution

LOT Batch No.

Product Expiry Date

CONT Content

Manufactured By

Read Instructions

Date of Manufacture

Storage Temperature

Keep Dry

REF Catalogue No.

Fragile

Keep away from sun light

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