Pariksha Biotech
A game changer in IVD

G6PDH Estimation Kit

High-Q G6PDH

(Quantitative/ Kinetic)

HIGH®
Command on Quality

Intended Use:
Kit for the quantitative determination of Glucose-6-phosphate dehydrogenase in human blood on photometric systems

Test Principle:
Glucose-6-phosphate dehydrogenase (G6PDH) catalyzes the first step in the pentose phosphate shunt, oxidizing glucose-6- phosphate (G-6-P) to 6-phosphogluconate (6-PG) and reducing NADP to NADPH:

G-6-P + NADP+ + G6PDH 6-PG + NADPH + 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:

NADP	2.0 mM
Maleimide	15 mM
Glucose-6-phosphate	1.05 mM
Magnesium salt	0.01 mM
Sodium azide	< 0.1 %
Buffer, stabilizers	

Reagent preparation and stability:
All the reagents are ready to use and stable till the expiry date mentioned on the labels:

Sample and 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 heparinized 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 **Hemoglobin 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 in to a clean glass tube:

Lysing Reagent:	1 ml
Whole Blood:	10 µl

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

Step -3 : Pipette in to clean glass test tube the following.

Substrate Reagent	0.5 ml
Buffer Reagent	0.5 ml
Hemolysate prepared above:	0.5 ml

Mix well and immediately aspirate in to the analyzer. After 60 seconds incubation, measure the change of optical density per 60 seconds during next 180 seconds against distilled water at 340 nm as follows.

Ao -	Exactly after 60 seconds
A1, A2, A3-	Exactly after every 60 seconds for 180 seconds.

Calculations:
Calculate the average change in absorbance per minute ΔAbs/min).

Activity of G6PDH (U/g Hb): $\Delta Abs \times \frac{4839}{Hb\ (gm/dl)}$

Activity of G6PDH ($U/10^{12}$ RBC's): $\Delta Abs \times \frac{48390}{RBC\ Count\ in\ Million}$

Programme 1 (System Parameters) For Testing of Random Clinical Samples

Reaction type	:	Kinetic
Reaction Direction	:	Increasing
Wavelength	:	340 nm
Flow cell temp	:	37°C
Zero setting with	:	Distilled water
Delay time	:	60 Sec
Measuring Time	:	180 Sec
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

System Parameters:

Reaction type	:	Kinetic
Reaction Direction	:	Increasing
Wavelength	:	340 nm
Flow cell temp	:	37°C
Zero setting with	:	Distilled water
Delay time	:	300 Sec
Measuring Time	:	300 Sec
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)

Factor Derivation:
G6PDH activity can be expressed as either U/g hemoglobin (Hb) or as $U/10^{12}$ erythrocytes (RBC):

G6PDH [U/g Hb] $= \Delta A/min \times \frac{100 \times 1.505 \times TCF}{0.005 \times 6.22 \times Hb\ (g/dl)}$

$= \Delta A/min \times \frac{4839 \times TCF}{Hb\ (g/dl)}$

Or: G6PDH ($U/10^{12}$ RBC) $= \frac{\Delta A/min \times 1.505 \times 10^{12} \times TCF.}{0.005 \times 6.22 \times (Nx10) \times 1000}$

$= \frac{\Delta A/min \times 48390 \times TCF}{N}$

100	=	Factor to convert activity to 100 ml
1.505	=	Total reaction volume (ml) (0.5 ml Buffer+0.5 ml Substrate+ 5µl Whole Blood in 0.5 ml Hemolysate)
0.005	=	Sample volume (ml) (Original Sample Volume 5µl Whole Blood in 0.5 ml Hemolysate)
6.22	=	Millimolar absorptivity of NADPH at 340 nm
Hb (g/dl)	=	Hemoglobin concentration for each specimen
TCF	=	Temperature Correction Factor (= 1 at 37°C)
($Nx10^6$)	=	Red cell count (red cells/mm3) for each specimen
N	=	Red cell count divided by 10^6
1000	=	Conversion of red cell count from mm3 to ml

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Linearity:
The maximum G6PDH activity which may be measured by this procedure is appr: (22.5 U/g Hb) 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 12.0 g/dl and a red cell count of 4.5 x 10^{12} /mm3, a G6PDH activity of 0.4 U/g Hb or 11 U/10^{12} RBC may be detected.

Method Comparison:
A comparison study between High-Q G6PDH (y) and a commercially available test (x) gave the following results:
y = 0.97 x + 0.07; r = 0.994

Normal Reference Values:

G6PDH Activity: (U/g Hb): 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/g Hb): Less than 6.4 at 37°C

(U/10^{12} RBC's): Less than 202 at 37°C

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

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 taken that the well washed glass tubes are used for testing.
5) Young Red Cells have a higher G6PD content than the older ones regardless of the genetic variant that is present.
6) If the enzymes have defective activity older cells are preferentially destroyed during mild to moderate hemolytic phase. Since reticulocytes released to replace lost cells have high enzyme levels, falsely elevated results may occur if blood is tested immediately after a hemolytic episode.
7) Normally the activity contributed by WBC, Platelets or serum is very small. Incases of severe anemia, leucocytosis 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, pp. 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, p 323, 1955.

Ordering Information:

Ref./Cat. No.	Pack Size	Presentation
P-G6P - 10	10 Tests	Three Reagents
P-G6P - 20	20 Tests	
P-G6P - 50	50 Tests	

Product Features

- **Liquid Stable, Ready to use Three Reagents (R1 + R2 + Lysing Reagent).**
- **NADP Analogues are used for better stability**
- **4 Minutes increasing Kinetic Reaction (60 Sec Delay+ 180 Sec Measuring) (For testing the Random Clinical Samples)**
- **10 Minutes increasing Kinetic Reaction(300 Sec Delay+ 300 Sec Measuring) (For the confirmation of deficient samples)**
- **Linearity: 25 (U/g Hb).**
- **Measuring Wavelength 340 nm**
- **Kinetic Factor : 4839 (U/g Hb) at 340 nm at 37° C.**
- **Whole blood is the specimen**
- **Available as multipurpose reagents.**

Symbols used with IVD devices

	Date of manufacture		Manufactured by
IVD	In vitro diagnostic device		Keep away from sunlight
	Do not freeze		This way up
	Use by (yyyy-mm-dd or mm/yyyy)	R	Reagent
CAL	Calibrator Material	LOT	Batch code
2°C 8°C	Temperature limitation (store at)	CONTROL	Control
	Consult instructions for use		Keep dry Keep away from rain
REF	Catalog Number		

eIFU Indicator

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SCAN TO EXPLORE MORE

Manufactured in India by :
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