LiquiMAX G6PDH (Quantitative/ Kinetic)

ORDERING INFORMATION

Ref./Cat. No.	Pack Size	Presentation
AVG6P1 - 10	10 ml	Three Reagents
AVG6P1 - 20	20 ml	

INTENDED USE

LiquiMAX G6PDH is an in-vitro diagnostic kit for the quantitative determination of Glucose-6-phosphate dehydrogenase in human blood on photometric systems.

PRODUCT FEATURES:

1. Liquid Stable, Ready to use Three Reagents (R1 + R2 + Lysing Reagent).
2. NADP Analogues are used for better stability
3. 4 Minutes increasing Kinetic Reaction (60 Sec Delay+ 180 Sec Measuring) (For testing the Random Clinical Samples)
4. 10 Minutes increasing Kinetic Reaction(300 Sec Delay+ 300 Sec Measuring) (For the confirmation of deficient samples)
5. Linearity: 25 (U/g Hb).
6. Measuring Wavelength 340 nm
7. Kinetic Factor : 4839 (U/g Hb) at 340 nm at 37° C.
8. Whole blood is the specimen
9. Available as multipurpose reagents.

CLINICAL SIGNIFICANCE :

Hemolytic anaemias or hemolytic episodes are related in most of the cases to enzyme deficiencies due to hereditary abnormalities. There are many screening nonspecific tests like osmotic fragility autohemolysis tests etc. Additional better screening tests for metabolic defects in red cell are to measure glucose consumption, lactate production or measure contribution of pentose phosphate pathway to metabolism. However, these tests being elaborate and difficult and still not being specific, it is better to identify these deficiences by enzyme assays. One of the common enzyme deficiencies for hemolytic episodes/ hemolytic anaemia is measurement of Glucose-6-Phosphate Dehydrogenase, by a quantitative enzyme assay. G-6-PD uses a potent inhibitor to prevent interference caused by 6-Phosphogluconate dehydrogenase.

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:

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

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.

STORAGE AND STABILITY:

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

KIT COMPONENTS

1. Buffer Reagent R1
2. Substrate Reagent R2
3. Lysing Reagent R3

COMPOSITION

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 RECONSTITUTION & STABILITY

Reagent are liquid stable no need for reconstitution.

When the reagent is stored properly at 2-8°C & the contamination avoided, it is stable up to the expiry date mention on the label & kit box.

MATERIAL REQUIRED BUT NOT PROVIDED

Laboratory Instrumentation, Spectrophotometer UV/VIS with thermostatic cuvette holder or clinical chemistry analyzer: semi auto, calibrated micropipettes, glass or high quality polystyrene cuvettes, test tube/rack, heating bath controls, saline.

REAGENT DETERIORATION

Discard any turbid reagent or blank reagent absorbance exceeds 1.4 at 340 nm against distilled water.

WARNING & PRECAUTIONS

- Reagent may contain some non reactive and preservative components. It is recommended to handle carefully, avoiding contact with skin and ingestion.
- Specimen should be considered infectious and handled appropriately.
- Contamination by soap or glycerol will affect this assay.
- Perform the test according to the general " Good Laboratory Practice" GLP

SPECIMEN & COLLECTION STORAGE

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.

A) 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/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)

B) 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
Reaction Direction	:	Increasing
Wavelength	:	340 nm
Flow cell temp	:	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)

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.

Buffer:	0.5 ml
Substrate:	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 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).

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

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

EXPECTED 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.

QUALITY CONTROL & CALIBRATOR

To ensure adequate Quality Control, the use of commercial reference control is recommended with each assay batch. Use of Quality control material checks both, the instrument and the reagent performances.

PERFORMANCE CHARACTERISTICS

1. Linearity

Linearity : 25 (U/g Hb)

2. Sensitivity/ Limit of Detection (LOD)

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.

3. Interferences

No significant interference was observed from Bilirubin up to 20 mg/dl (Both conjugated and unconjugated Bilirubin) Hemoglobin up to 50 mg/dl,Lipemia as Triglycerides up to 2000 mg/dl, Ascorbic acid up to 50 mg/dl.

4. Precision:

Intra-Assay

Sample	Mean (u/g)	SD (u/g)	CV%
Control serum 1	8.17	0.13	1.59
Control serum 2	14.88	0.32	2.15
Control serum 3	22.77	0.58	1.95

Inter-Assay

Sample	Mean (u/g)	SD (u/g)	CV%
Control serum 1	8.21	0.033	1.49
Control serum 2	14.92	0.035	1.32
Control serum 3	22.82	0.031	0.80

5.method Comparison

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

LIMITATIONS

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.

WASTE DISPOSAL

Reagents must be disposed off in accordance with local regulations.

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.

Symbols Used on Pack

REF	Catalogue Number		Warning/Caution
LOT	Batch No.	IVD	In vitro diagnostic device
	Manufacturing Date		Storage Limit
	Expiry Date		Consult instruction for use
	Manufacturer		Keep away from sunlight
	Keep Dry		Do not use if package is damaged
	Contains sufficient no. of test		

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