PATHOZYME DIAGNOSTICS

LIQVIPATH G6PDH

(QUANTITATIVE / KINETIC METHOD)
CAT NO.: GPK

Reagent kit for quantitative estimation of G6PDH in Human Blood.

DIAGNOSTIC SIGNIFICANCE:

Hemolytic anemias 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 auto hemolysis 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 deficiencies by enzyme assays.

One of the common enzyme deficiencies for hemolytic episodes / hemolytic anemia 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:

The enzyme Glucose-6-Phosphate Dehydrogenase present in the Red Blood Cells is extracted by lysing the cells using a natural detergent. The extracted enzyme oxidases Glucose-6-Phosphate to 6-Phosphogluconate and simultaneously reduces co-enzyme NADP to NADPH giving increase in absorbance at 340 nm.

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

SPECIMEN COLLECTION:

Fresh whole blood is the specimen required. Collection of blood by using any one of the anticoagulants such as EDTA, citrate, Oxalate or Heparins recommended.

Determine the **Hemoglobin content** of the whole blood or the **RBC count** prior to lysis of the cells.

KIT PRESENTATION:

PACK SIZE	12 Test	24 Test
R1-G6PD (Substrate Reagent)	1 X 6 ml	1 X 12 ml
R2-G6PD (Buffer Reagent)	1 X 6 ml	1 X 12 ml
R3-G6PD (Lysing Reagent)	1 X 12 ml	1 X 25 ml

REAGENT PREPARATION AND STABILITY:

All reagents are ready to use and stable at 2-8°C until the expiry date stated on the label.

PRECAUTIONS:

1. **R3-G6PD (Lysing Reagent) must be cold (2-8°C) before use.**
2. **Heparin sample gives unreliable count after 2 days and in such** cases the results are best reported in Hb Concentration.
3. Copper and Sulphate ions inhibit G6PDH activity and care should be taken that the well washed Test Tube for assay.
4. In cases of severe Anemia, Leukocytosis or very low G6PDH Levels, the use of sample after removing the Buffy Coat is recommended.

ASSAY PARAMETERS:

Reaction	**: Kinetic**	**Sample Volume**	**: 10 µl**
Wavelength	**: 340 nm**	**R1 + R2 Volume**	**: 500 µl + 500 µl**
Flow Cell Temp.	**: 30°C or 37 °C**	**Factor**	**: 4839/Hb or 48390/RBC**
Initial Delay	**: 60 Sec**	**Reaction Slope**	**: Increasing**
Interval Time	**: 60 Sec**	**Zero Setting**	**: Distilled Water**
Read Time	**: 180 Sec**	**Linearity (in Hb)**	**: 25 U/g Hb**
No. of Reading	**: 03**	**Linearity (in RBC)**	**: 698 U/10^{12}RBC**

Note: If G6PDH activity or the absorbance change per minute will be very low. In such cases adapt the change in above assay parameter: **Initial Delay Time 300 sec** and **No. of Reading 06**.

PROCEDURE:

Step-1: Determine the **Hemoglobin content** OR the **RBC count** of the whole blood prior to perform the G6PDH assay.

Step-2: Preparation of Hemolysate:

Take into clean Test Tube:

Lysing Reagent (Use must be cold) : 1 ml
Whole Blood : 10 µl

Mix well, allow 10 minutes at RT. **This is Hemolysate for assay**.

Step-3: Pipette into clean Test Tube the following:

R1-G6PD (Substrate Reagent)	500 µl (0.5 ml)
R2-G6PD (Buffer Reagent)	500 µl (0.5 ml)
Above Prepared Hemolysate	500 µl (0.5 ml)

Mix & aspirate immediately and read **first** absorbance of test exactly at 60 seconds and then, **second, third** and **fourth** at an interval of 60 seconds at 340 nm. Determine the mean change in absorbance per minute. (ΔA/min) and calculate the test results.

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(P.T.O.)

Expiry Date | In-Vitro Diagnostics Use | Storage | Mfg. Date | Batch Number | Catalogue Number | See Package Insert

PATHOZYME DIAGNOSTICS
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CALCULATION:

G6PDH Activity (U/g Hb) = $\frac{\Delta A/min \times 4839}{Hb\ (gm/dl)}$

G6PDH Activity (U/10^{12}RBC) = $\frac{\Delta\ A/min \times 48390}{RBC\ Count\ in\ Million}$

FACTOR CALCULATION:

G6PDH (U/gHb) = $\frac{\Delta A/min \times 100 \times 1.505 \times TF}{0.005 \times 6.22 \times Hb(gm/dl)}$

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

G6PDH (U/10^{12}RBC) = $\frac{\Delta A/min \times 10^{12} \times 1.505 \times TF}{0.005 \times 6.22 \times (N \times 10^{6}) \times 1000}$

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

Where

100	= Convert activity to 100 ml
1.505	= Total Volume (1ml Rgt + 5µl Whole Blood in 0.5ml Hemolysate)
0.005	= Sample Volume (Original 5µl Whole Blood in 0.5ml Hemolysate)
6.22	= Millimolar absorptivity of NADPH at 340 nm
Hb(g/dl)	= Hemoglobin Concentration of sample
TF	= Temperature Correction Factor (= 1 at 37ºC /1.39 at 30^0C)
(Nx10^6)	= Red Cell Count (Red Cells/mm^3) of sample
N	= Red Cell Count divided by 10^6
1000	= Conversion of Red Cell Count from mm^3 to ml

NORMAL VALUES:

At 30ºC : 4.6 – 15.0 U/gHb **OR** 146 – 417 U/10^{12}RBC
At 37ºC : 6.4 – 20.0 U/gHb **OR** 202 – 558 U/10^{12}RBC
Each laboratory should establish its own reference range.

DEFICIENT VALUES:

At 30°C : Less than 4.6 U/gHb **OR** 146 U/10^{12}RBC
At 37ºC : Less than 6.4 U/gHb **OR** 202 U/10^{12}RBC

LINEARITY:

This method is linear up to **25 U/gHb or 698 U/10^{12}RBC**. For values above linearity repeat assay using **5µl** blood as sample and multiply results by 2.

REFERENCES:

1. Kachmar J. F., Moss. D. W.,: Enzymes. In Fundamentals of Clinical Chemistry Ed. by N. W. Teitz, Saunders Philadelphia 1976 pp 666-672.
2. Burtis, C.A.,Ashwood, E.R. Tietz Clinical Chemistry, W.B. Saunders, Philadelphia, pp 1645-1650, 1999.

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