G6PDH LYPHO
(Quantitative/Kinetic Method)

Intended Use
Reagent Kit for quantitative estimation of G6PDH in erythrocytes.

Reagent Provided
1. G6PDH (Co-Enzyme – Substrate)
2. G6PDH (Buffer Reagent)
3. G6PDH (Lysing Reagent)

Diagnostic Significance
G-6-PD deficient subjects are prone to hemolytic episodes and hemolytic anaemia if treated with certain antimalarial or sulfa drugs. The screening for the deficiency is one of the first preventive step to avoid these hemolytic episodes and anaemia. The estimation will indicate deficiency better in males than in females.

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 oxides Glucose-6-Phosphate to 6-Phosphogluconate and simultaneously reduces co-enzyme NADP to NADPH giving increase in absorbance at 340 nm.
Enzymatic determination of G6PDH activity is based on the following reaction.

Glucose-6-Phosphate +NADP $\xrightarrow{\text{G6PDH}}$ 6-Phosphogluconate +NADPH

Components & Concentration of Reagents

Reagent	Component	Concentration
Active Ingredients	Glucose-6-Phosphate	0.5 mmol/L
	$NADPNa_2$	0.75 mmol/L
	Buffer	100 mmol/L
	Detergent	1 mmol/L
	pH7.5±0.1 at 25°C	

Reagent Storage & Stability
G6PDH reagents are stable until the expiry date stated on the label. Reconstituted reagent is stable for 7 days at 2-8°C.

Preparation of Working Reagent
Add 1.1 ml buffer (2G6PDH) to the Vial labeled 1G6PDH. Mix well and use after five minutes.

Specimen Collection & Preservative
Fresh whole blood is the specimen required. Collection of blood by using any one of the anticoagulants such as Citrate, Oxalate or Heparin is recommended.

Preparation of Red Cell Hemolysate
Wash 0.1 ml of whole blood with 2 ml aliquots of physiological saline (0.9%) 3 times and suspend the washed, packed and centrifuged erythrocytesin precooled 0.5 ml of 3G6PDH (lysing Reagent). Mix well and keep in the refrigerator (2-4°C) for atleast 15 minutes and maximum for 2 hours. Centrifuge the lysate at 3000 r.p.m for 5 minutes prior to use.

Precaution
G6PDH is for IN-VITRO diagnostic use only.
Reagent contains Sodium Azide.
Do Not Ingest
The Lysing Reagent (3G6PDH) should be used cold (0-8°C) to avoid theDecrease in enzyme activity.

Assay Guidelines for Analyzers

Reaction Type	Kinetic
Wavelength	340 nm
Slope of Reaction	Increasing
FlowCellTemperature Setting	30°C
Zero Setting With	Distilled Water
Delay Time	180 sec
Interval Time	60 sec
No. of Readings	4
Working Reagent Volume	1.0 ml
Sample Volume	25 µl (0.025 ml)
Factor	3601 (When Hemoglobin is Measured)
	36013 (When RBC Count is measured)
Linearity	Up to (22.5 U/g Hb) or (628 $U/10^{12}$ RBC'S)

Test Procedure
Determine the Hemoglobin content of the whole blood and the RBC count prior to lysis of the cells.

Pipette Into Test Tubes	Test
Working Reagent	1.0 ml
Hemolysate	0.025 ml

Mix and aspirate. After the initial delay of 180 sec., record the absorbance of test at the interval of one minute for the next 3 minutes at 340 nm. Determine the mean change in absorbance per minute and calculate test results.

Calculation
(i) PDH Activity ($U/10^{12}$ RBC)

$$\Delta A/Min x = \frac{224 x 10^{12}}{6.22 x N x 10^{6} x 1000}$$

$$\Delta A/Min x = \frac{36013}{N}$$

Where
224 = Total assay volume to sample volume
10^{12} = Factor for expressing activity in 10^{12} cells

6.22 = Milimolar absorptivity of NADPH at 340 nm.
$Nx10^6$ = Number of erythrocytes/cmm
1000 = Conversion of Cell Count from Count per cmm to count per ml

(ii) G6PDH Activity (U/gHb)

$$\Delta A/Minx=\frac{224x100}{6.22xHb(g/dl)}$$

$$\Delta A/Minx=\frac{3601}{Hb(g/dl)}$$

Where
100 =Factor to convert to 100 ml
224 = Total assay volume to sample volume
6.22 = Milimolar absorptivity of NADPH at 340 nm.
Hb(g/dl) = Hemoglobin Concentration

Example

Assay of specimen which had a red cell count of $4.6x10^6/mm^3$ and a hemoglobin concentration of 15.2 g/dl result in ΔA/min at 30°C of 0.028.

$$G6PDH(U/10^{12}RBC)=\frac{0.028\ x\ 36013}{4.6}=219$$

$$G6PDH(U/gHb)=\frac{0.028\ x\ 3601}{15.2}=6.63$$

Normal Values

G6PDH Activity (at 30°C) 146-376 $U/10^{12}$ RBCs
4.6 – 13.5 U/g Hb

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.

Notes

If the G6PDH activity is very low, measure the absorbance change for 5 minutes after addition of buffered substrate and divide by 5 to obtain ΔA/minute and calculate the test results. Use the formula given under "TEST RESULTS".

References

1. Kachmar J.F. Moss S.W. Enzymes. In Fundamentals of Clinical Chemistry Ed. By N.W. 2.Teitz. Saunders Philadelphia. 1976 p.p 666-672.
2. Gross R.T, Hurwitz R.E., Marks P.A. Red Cell Glucose-6-Phosphat dehydrogenase deficiency. J. Clin.Invest (1978) 37.176.

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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