NS BIOTEC

GPDH

REF: G6-MK-1001 (10x1ml)

Reagent kit for quantitative estimation of G6PDH in Human Blood.

PRINCIPLE:

The enzyme Glucose-6-Phosphate Dehydrogenase present 1n the Red Blood Cells JS extracted by lysing the cells using a natural detergent. The extracted enzyme oxidises Glucose-6-Phosphale to 6-Phosphogluconate and simultaneously reduces co-enzyme

NADP to NADPH giving increase in absorbance at 340 nm.

G-6-P + NADP GS DH> 6•Phosphog!uconate + NAOPH + H'

SPECIMEN COLLECTION:

Fresh whole blood is the specimen required. Collection of blood by using any one of the antlcoagu!ants such as EDTA, citrate, Oxalate or Hepanms recommended.

Determine the Hemoglobin content of the whole blood and the

RBC count prior to lysts of the cells.

REAGENT PREPARATION AND STABILITY:

All reagents are ready to use and stable at 2-s•c until the expiry date stated on the label.

ASSAY PARAMETERS:

Reaction: Kinetic	Sample volume : 10 µl
Wavelength 340 nm	Reagent volume : 500 µl
Flow cell Temp.: 37 °C	Factor : 4839/HB or 48390/RBC
Initial delay : 60 sec	Reaction slop : increasing
Interval time : 60 sec	Zero setting :Dist. water
Read time : 180 sec	Linearity (in Hb) : 25 U/g Hb
No. of reading : 4	Linearity (in RBC) : 25 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: 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: Preparatlon of Hemolysate: Take into clean Test Tube:

Lysing Reagent: 1.0 ml

Whole Blood : 10 µI

Mix well allows 10 minutes at RT. This Is Hemolysate for assay.

Ste -3: Piptte Into clean Test Tube the following

G6PD(Substrate Reagent 1)	250 µl
G6PD(Buffer Reagent 2)	250 µl
Above prepared hemolysate	250 µl

Mix & aspirate immediately and read 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. {△A/ min) and calculate the test results.

Factor Calculation:

$$\text{G6PDH (U/gHb)} = \frac{\triangle A/\text{min} \times 4839}{\text{Hb(gm/dl)}}$$

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

DEFICIENT VALUES:

At 37°C : 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.

Precautions:

1. Heparin sample gives unreliable count after 2 days and in such cases the results are best reported in Hb Concentration.

2. Copper and Sulphate ions inhibit G6PDH activity and care should be taken that the well washed Test Tube for assay.

3. !n cases of severe Anemia, Leucocytosis or very low G6PDH levels, the use of sample after removing the Buffy Coat 1s recommended.

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