G6PDH Kit

Kinetic Method

KIT NAME	KIT SIZE	CAT. NO
NBIO G6PDH Kit	25 ml	GBG6P0125M

GENUINE BIOSYSTEM

CLINICAL SIGNIFICANCE

Glucose-6-Phosphate-Dehydrogenase (G6PD) deficiency is one of the most common human enzyme deficiency in the world. During G6PD deficiency, the red cells are unable to regenerate reduced Nicotine adenine dinucleotide phosphate (NADPH), a reaction that is normally catalyzed by the G6PD enzyme. Since the X chromosome carries the gene for G6PD enzyme, this deficiency mostly affects the males.

The two major conditions associated with G6PD deficiency are hemolytic anaemias and neonatal jaundice, which may result in neurological complications and death. Screening and detection of G6PD deficiency helps in reducing such episodes, through appropriate selection of treatment, patient counselling and abstinence from disease precipitating drugs such as anti malarials and other agents.

PRINCIPLE

Glucose 6 phosphatase dehydrogenase catalyzes the first step in the pentose phosphate shunt, oxidizing glucose-6- phosphate to 6 phosphogluconate and reducing NADP to NADPH. The gbpl procedure is a spectrophotometric method based on the following reaction

G6P+ NADP+ ----------→ 6-PG + NADP + H+ NADP is reduced by G-6PDH in the presence of G6P. The rate of formation of NADPH is proportional to the G-6-PDH activity and is measured spectrophotometrically as an increase in absorbance at 340 nm Production of a second molar equivalent of NADP by erythrocyte 6-phosphogluconate dehydrogenase.

6-PG+NADP+ ----------→ Ribulose-5-Phosphate + NADPH +H+ CO2.

Is prevented by use of maleimide and inhibitor of 6-PGDH.

KIT CONTENTS:

Name	Pack
R1-G6PDH Reagent	1 x 25 ml
R2-Substrate Reagent	1 x 50 ml

SPECIMEN COLLECTION AND PRESERVATION

Whole blood collected in EDTA, Heparin or ACD is satisfactory. Red cell-G-6-PDH is stable in whole blood for one week refriferated but is unstable in red cell hemolysate.Freezing of blood is not recommended .Since activity is reported in terms of number of red cells or grams of hemoglobin.The red cell count or hemoglobin concentration should be determined prior to performing of G-6-PDH assay. The integrity of erythrocytes collected in ACD is preserved even after prolonged storage so that obtaining accurate red cell counts poses no problem.However red cell counts on specimens collected in heparin become unreliable after about 2 days.Thus for heparinized sample results are best reported in terms of hemoglobin concentration.

Both copper, which completely inhibits the enzyme at a concentration of 100 mmol/L and sulfate ions (0.005 mol/L) will decrease observed values of G-6-PDH activity,Certain drugs and other substances are known to influence circulating levels of G-6-PDH .

Reticulocytes have higher G-6-PDH levels than mature red cells.Therefore it is not recommended that assay be performed after a severe hemolytic crisis, since G-6-PDH levels appear falsely elevated. Under those conditions detection of deficiency may require family studies. Testing may be more helpful after the level of mature red cells has returned to normal. Under normal circumstances activity contributed by leucocytes, platelets and serum is relatively small. However in cases of extreme anemia, grossly elevated white counts or very low levels of red cell G-6-PDH activity, the contribution to the total made under these circumstances may be significant. See"use of Buffy coat-free samples" section.

REAGENT PREPARATION

Reagents are supplied, ready To Use

REAGENT STORAGE AND STABILITY

Store Reagent at 2-8^0 C. Reagents are stable until expiration dates shown on the labels.

MANUAL ASSAY PROCEDURE

The temperature of the reaction mixture should be maintained at 30 °c or some other constant temperature.

Reagent	ML (µl)
R1 – G6PDH Reagent	1.0 ml(1000 µl)
Sample	0.01 ml(10 µl)
Mix well and incubate for 5 mins at R.T & add	
R2- Substrate Reagent	2.0 ml (2000 µl)
Mix well and After 30 secs read the absorbance (A0) & repeat the absorbance reading after every 1,2,3,4,&5 min. Calculate Mean absorbance Change per min. (ΔA per min)	

CALIBRATION:

The procedure is standardized on the basis of the millimolar absorptivity of NADPH which is 6.22 at 340 nm. The oxidative conversion of G6P by G6PDH leads to reduction of NADP to NADPH on a molar equivalent basis. Measurement of the rate of increase in absorbance at 340 n serves to quantitate enzymatic activity. The maximum G-6-PDH activity which may be measured by this procedure is approximately 650 U/10^{12} RBC or 19.5 U/gHb

CALCULATIONS

G6PDH activity is expressed as U/10^{12} erythrocyte or U/g hemoglobin (Hb)

$$\text{G6PDH (U/10}^{12}\text{ RBC)} = \Delta A \text{ per min} \times \frac{48390}{N} \times TCF$$

Where N= Red cell count divided by 106

TCF = temperature correction factor (1 at 30°c)

$$\text{G6PDH (u/g Hb)} = \Delta A \text{ per min} \times \frac{4839X}{\text{Hb(g/dL)}} \times TCF$$

TEMPERATURE CONVERSION FACTORS

Assay Temperature	Desired Reporting Temperature 25°C	30°C	37°C
25°C	1.00	1.32	1.82
30°C	0.76	1.00	1.39
37°C	0.55	0.72	1.00

Example :

Assay of a specimen which had a red cell count of 4.6 x/mm3 and a hemoglobin concentration 15.2 g/dL resulted in ΔA per min at 30°c of 0.026.

G6PDH ($U/10^{12}$ RBC) = 0.026 x $\frac{48390 \times TCF}{4.6}$ = $295 U/10^{12}$ RBC

G6PDH (U/g Hb) = 0.026 x $\frac{4839}{15.2}$ x TCF = 8.9U/g Hb

NOTE : If ΔA per min is greater than 0.060 repeat determination (3) using 5 µl blood and multiply results by 2.

USE OF BUFFY COAT-FREE SAMPLE

Under normal circumstances g6PDH activity contributed by leucocytes, platelets an serum is relatively small. However as reported by Echler and others more accurate measurement of G6PDH activity specially in presence of anemia and or leucocytosis can be achieved by using buffy coat free blood samples for assay. Thus in case of a borderline value obtained with whole blood it may be warranted to repeat the assay on a buffy coat free sample.

UNIT DEFINITION

One international unit is that amount of G6PDH activity that will convert 1 micromole of substance per minute under the conditions specified in the insert. Activity may be expressed in terms of either a standard no of cell or amount of hemoglobin.Since it is preferred despite the fact that it is believed by some that red cell counts are subject to considerable uncertainity. Hemoglobin concentration may be determined with greater accuracy but the amount of hemoglobin contained in a cell is under separate genetic control and may wary independently of G6PDH activity.

REFERENCE VALUES

The following range of G6PDH values measured at 30°C was obtained in our laboratory for 100 clinically healthy males and females

G6PDH Activity
146 - 376 ($U/10^{12}$ RBC)
4.6 - 13.5 (U/g Hb)

Values for newborn may range somewhat higher.
It is recommended that each laboratory establishes its own normal range.
It has been determined that G6PDH deficiency in red cells is the basis for certain drug induced hemolytic anemias.This type of susceptibility to drug induced hemolysis is often called “primaquine sensitivity” because studies which led to its characterization were made during investigations of the hemolytic properties of this antimalarial compound.

SYSTEM PARAMETERS

Method	Kinetic
Wavelength	340 nm
Zero Setting	Distilled water
Temperature Setting	37°C
Incubation Temperature	37°C
Delay Time	30 secs
Read Time	300 secs
No. of Reading	5
Interval Time	60 secs
Sample Volume	0.01ml (10µl)
Reagent Volume	3.0ml (3000µl)
Standard Concentration	-
Units	u/g Hb
Factor	4839
Reaction Slope	Increasing
Linearity	19.5 u/g Hb

IVD CE

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