

Quantitative Determination of G6PDH in Whole Blood.
Only for *In Vitro* Diagnostic use

ORDER INFORMATION

REF	Cont.
G6PDQLS 50	1 X 50 ML

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 accucare procedure is a spectrophotometric method based on the following reaction: $G6P + NADP^+ \rightarrow 6-PG + NADP + H^+$. 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.

$6-PG + NADP^+ \rightarrow$ Ribulose-5-Phosphate + NADPH + H⁺ + CO₂.
Is prevented by use of maleimide and inhibitor of 6-PGDH.

REAGENT COMPOSITION

Reagent 1 : Assay Reagent
Reagent 2 : Substrate reagent

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 refrigerated 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

FULLY AUTOMATED PROCEDURE

Lysate Preparation: 30 µl of Whole blood sample mix with 270 µl Distilled water mix well and wait for 10 to 15 minutes

AUTOMATED PARAMETERS	
Wavelength	340 nm
Measurement	Against Distilled Water
Cuvette	1 cm light path
Reaction Temperature	30°C
Reaction Type	Kinetic
Reaction Direction	Increasing
Sample Volume (Lysate)	50µl
Assay Reagent Volume	250µl
Substrate Reagent Volume	500µl
Delay/Lag/time	300 sec.
Interval time	60 sec.
No. of Readings	05
Factor	4839
Low Normal at 37°C	4.6 u/g Hb
High Normal at 37°C	13.5 u/g Hb
Linearity at 37°C	19.5 u/g Hb

MANUAL ASSAY PROCEDURE

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

Addition Sequence	ML (µl)
Assay Reagent	500 µl
SAMPLE	5 µl
Mix well and incubate for 5 mins at 30°C & Immediately Add	
Substrate Reagent	1.0 ml (1000 µl)
Mix well and After 5 minutes read the absorbance (A ₀) & 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 nm serves to quantitate enzymatic activity. The maximum G-6-PDH activity which may be measured by this procedure is approximately 650 U/10¹² RBC or 19.5 U/gHb

CALCULATIONS

G6PDH activity is expressed as U/10¹² erythrocyte or U/g hemoglobin (Hb)

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

Where N= Red cell count divided by 106
TCF = temperature correction factor (1 at 30°C)

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

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.

$$\begin{aligned} \text{G6PDH (U/10}^{12} \text{ RBC)} &= 0.026 \times \frac{48390}{4.6} \times \text{TCF} = 295 \text{ U/10}^{12} \text{ RBC} \\ \text{G6PDH (U/g Hb)} &= 0.026 \times \frac{4839}{15.2} \times \text{TCF} = 8.9 \text{ U/g Hb} \end{aligned}$$

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 and 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 uncertainty. Hemoglobin concentration may be determined with greater accuracy but the amount of hemoglobin contained in a cell is under separate genetic control and may vary 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.