

BioMed-G6PD

Cellular enzyme determination reagents



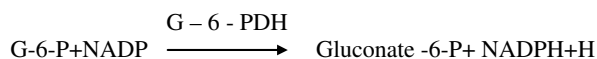
REF: G6P12205 (5 test)
G6P12210 (10 test)

INTENDED FOR USE:

For the quantitative determination of G6PD.

PRINCIPLE:

G6PDH in the **RBC's** is released by a lysing agent present in the reagent. The **G6PDH** released catalyzes the oxidation of Glucose 6 phosphate with the reduction of **NADP** to **NADPH**. The rate of reduction of **NADP** to **NADPH** is measured as an increase in absorbance which is proportional to the G6PDH activity in the sample.



SPECIMEN COLLECTION:

Fresh whole blood sample collected in EDTA, Heparin or ACD (Acid-Citrate-Dextrose). Red Cell G6PDH in whole blood is reported to be stable for 7 days at 2-8°C, but is unstable in hemolyzates. Freezing is not recommended

REAGENT COMPOSITIONS :

G6PDH Activity (U/g Hb.): 4.6 to 13.5 at 30°C / 6.4 to 18.7 at 37°C
(U/10¹² RBC's): 146 to 376 at 30°C/ 202 to 522 at 37°C
it is recommended that each laboratory establish its own normal

Contents	5 x 1 test	5 x 5 tests
R1 : G6PDH Reagent	5 x 1 ml	5 x 5.5 ml
R2 : Starter Reagent	10 ml	50 ml

PACKAGE: Collection and storage.

Contents are stable at 2-8°C till the expiry date mentioned on the labels.

PRECAUTIONS & WARNING :

Avoid pipetting with mouth.

The preparation, according to current regulation, is classified as not dangerous.

The total concentration of non active components (preservatives, detergents, stabilizers) is below the minimum required for citation.

Anyway handle with care, avoid ingestion, avoid contact with eyes, skin and mucous membranes.

The samples must be handle as potentially infected from HIV or Hepatitis.

REAGENT PREPARATION & STABILITY :

Reconstitute G6PDH reagent (R1) with D.W. as per the volume mentioned on the label. This working reagent is stable for 6 hours at R.T. and at least 5 days when stored at 2-8°C.

The Starter Reagent (R2) is ready to use

REQUIRED MATERIALS NOT PROVIDED:

General Laboratory Equipment and instrumentations.

PROCEDURE:

Wavelength : 340 nm
Temperature : 30°C / 37°C
Optical path : 1 cm

Addition	T
Sequence	(ml)
G6PD Working Reagent (R1)	1.0
Whole Blood	0.01
Mix well & incubate for 5 -10 min. at R.T. and add	
Starter Reagent	2.0

Mix well & incubate for 5 min. at 30°C / 37°C and read the initial absorbance A₀ & repeat the absorbance reading after every 1, 2, & 3 minutes. Calculate the mean absorbance change per minute (Δ A/ min.).

If the G6PDH activity is very low, the absorbance change per minute will also be very low. In such cases read the initial absorbance A₁ and read another absorbance A₂ exactly 5 min. later. Calculate the mean absorbance change per minute (Δ A/ min.).

$$\Delta A / \text{min.} = \frac{A_2 - A_1}{5}$$

CALCULATION:

$$\text{G6PDH Activity (U/10}^{12}\text{ RBC)} = \Delta A \times \frac{47780}{\text{RBC Count in million}}$$

$$\text{G6PDH Activity (U/g Hb)} = \Delta A \times \frac{4778}{\text{Hb (g/dl)}}$$

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

EXPECTED VALUE:

System Parameters			
Reaction	Kinetic	Interval	60
Wavelength	340 nm	Sample Vol.	0.01 ml
Zero Setting	D.W.	Reagent Vol	3.00 ml
Incub. Temp	37°C	Standard	-----
Incub. Time	5 min	Factor	47780/RBC count
	+ 5 min.		4778/Hb
Delay Time	30 sec	React. Slope	Increasing
Read Time	180 sec	Linearity	-----
No. of read	4	Units	-----

WASTE DISPOSAL:

The disposal of the product must be in accordance with local regulation concerning waste disposal.

REFERENCES:

WHO, Tech. Rep. Ser. No. 366, 1967.

Diagnostic Hematology by Rodak, W.B. Saunders, 1995 Ed.:218.

Jacques Wallach, Interpretation of Diagnostic Tests, V Edition, page 315.

Tietz, Clinical Chemistry, Saunders (1986), page no, 1501-12.

Varley, H. Practical Clinical Biochemistry, V Edition, 729 -71






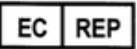



LIMITATIONS :

Since the activity of G6PDH is reported in Hb. Concentration or RBC count the same should be determined before performing the assay. RBCs are well preserved when collected in ACD(Acid-Citrate-Dextrose) and such samples give an accurate count, for samples collected in heparin counts become unreliable after 2 days and in such cases results are best reported in Hb concentration.

Copper and Sulphate ions inhibit the G6PDH activity, hence use of good quality D.W for reconstitution of R1 and use of properly cleaned glassware is essential.

Young red cells have a higher G6PD content then the older ones, regardless of the genetic variant that is present. If the enzymes have defective activity, older cells are preferentially destroyed during mild to moderate hemolytic phase. Since reticulocytes released to replace lost cells have high enzyme levels, falsely elevated results may occur if blood is tested immediately after a hemolytic episode.

Normally the activity contributed by WBC, platelets or serum is very small. In cases of severe anemia, leucocytosis, or very low G6PDH levels, the use of a sample after removing the Buffy Coat is recommended.

	Consult Instructions for Use
	Caution, consult accompanying Documents
	In Vitro Diagnostic Medical Device
	Temperature limitation
	Manufacturer
	Authorized Representative in the European Community
	Catalogue number
	Batch code
	Use by

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