GLUCOSE-6-PHOSPHATE DEHYDR. (G6PDH)

KINETIC UV DETERMINATION IN SERUM, PLASMA and ERYTHROCYTES
For in vitro diagnostic use only

Kit: 20 x 3 mL **Cod. G6P8905**

SUMMARY

The G6PDH is an enzyme of the Hexose-Monophosphate Pathway, and the only related to primary blood disorders.
G6PDH catalyzes the reaction from Glucose-6-P to Phosphogluconate in the RBC; G6PDH presents several genetic variants.
The strong reduction of activity of G6PDH in a few genetic variants may cause from mild to severe hemolytic disease and crisis, sometimes with fatal outcome.

PRINCIPLE

The Glucose-6-Phosphate Dehydrogenase (G6PDH) catalyzes the first step in the pentose phosphate shunt, oxidizing Glucose-6-phosphate (G-6-P) to 6-phosphogluconate (6-PG) and reducing NADP to NADPH.
The increase of absorbance of NADPH, for reduction of NADP, is proportional to the activity of the G-6-PDH in the sample.

REAGENTS

Components of the kit:		**Cod. G6P8905**
***REAGENT 1 (liquid)**		**1 x 20 mL**
***REAGENT 2 (lyo)**		**20 x 1 mL**
***REAGENT 3 (liquid)**		**1 x 40 mL**
Good Buffer modif.	>20 mmol/L	
G-6-P	>0.1 g/L	
NADP	>0.19 mmol/L	
Activators, Stabilyzers		

STABILITY: the reagents, at 2-8°C, are stable up to the expiry date shown on the package **if not contamined during handling**.

AUXILIARY REAGENTS (Not supplied with this kit)

1)To assure proper calibration performance, we suggest following kit:
-G6PDH calibrator 3 x 3 mL Cod. G6CAL (see the insert)

2)To assure proper test performance, we suggest following kits:
- LEVEL 1 ENZYME Control 5 x 3 mL Cod. BEE1005
- LEVEL 2 ENZYME Control 5 x 3 mL Cod. BEN2005
who contains a few enzymes in different ranges (see the inserts).

3) To prepare the **emolysate to use ONLY with discrete analyzer**, we suggest following kit:
- RED CELL LYSING Reagent 4 x 25 mL **Cod. B1129**
(see the related insert).

PREPARAT. OF THE WORKING REAGENT (*R1+*R2)

***KIT 20 x 3 ml (Cod. G6P8905)**
Add 1 ml of *Reagent 1 to one vial of *Reagent 2. Mix gently until dissolution.
STABILITY: 5 days at 2-8°C.

Mix kindly and let the working reagent reaches the working temperature before use. Close immediately after handling.

PREPARATION OF THE *REAGENT 3

***Reagent 3** **REAGENT READY TO USE.**

Let the reagent s reach the working temperature before use.
Mix kindly before use.
Close immediately after handling.

The Reagents have to be used properly, to avoid contamination. Incompetent handling will keep us harmless from any responsibility.

SAMPLE (see also NOTE)

• No haemolyzed fresh serum or plasma EDTA or heparin.
• Whole blood collected with EDTA, heparin or ACD (Acid-Citrate-Dextrose).
• Red cell G6PDH is stable in whole blood for 1 week at 2-8°C, **but is unstable in Red Cell hemolysate. Freezing of blood is not recommended. (see References 2. et 4.)**
• Since activity is reported as number of Red Cells or grams of Hemoglobin, these two ones have to be det. before G6PDH assay. The most accurate red cell counts in the time is with erythrocytes in ADC, due to longer integrity of erythocytes in it than in other ones. For sample in heparin, results are best reported in terms of hemoglobin concentration **(see References 5.)**

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PROCEDURE FOR SERUM-PLASMA (see Note 8.)

• Wavelength:	340 nm (334-365 nm)
• Pathlength:	1 cm
• Reading:	against air or distil. water
• Temperature:	37°C
• Method:	kinetic
• Reaction:	10+2+5 minutes
• Linearity:	till ΔA/min. 0,060
• Sample/Reagent:	1/100/200

Let reagent reaches the working temperature before using.

Pipette into a test tube or cuvette labelled:
S: Sample, ST: Calibrator/Standard/Control :

	ST	S
Working Reagent (*R1+*R2)	1000 µl	1000 µl
Calib./Standard/Control	10 µl	----
Sample	----	10 µl

Mix kindly and incubate for about 5-10 min. at 37°C. Add:

*Reagent 3	2000 µl	2000 µl

Mix kindly. Exactly 2 min. after, make the **FIRST** reading of control (Astd1) and sample (As1).
Repeat the **SECOND** readings after 5 min. for control (Astd2) and sample (As2).
Determine the absorbance/minute for Control (ΔAstd/min) and Sample (ΔAs/min), as : (**SECOND** readings – **FIRST** readings) / 5

CALCULATION

Insert the means found in the following formula:

$$\text{G6PDH (U/L 37°C)} = \text{Control value} \times \frac{\Delta\text{As/min. Sample}}{\Delta\text{Astd/min. Control}}$$

REFERENCE VALUES

0,00 – 0,18 U/L (37°C)

It is suitable that every laboratory determine its reference values.

PERFORMANCE CHARACTERISTICS

These performance characteristics was determined using a spectrophotometer or analyzers typically found in clinical laboratories, under the stated assay conditions.

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PROCEDURE FOR ERYTHROCYTES

Preliminary

Before to test G6PDH it is necessary to determine one of these things:
1) the number of erythrocytes (RBC) to express G-6-PDH activity as U/$10^{\wedge 12}$ erythrocytes (RBC), or
2) the concentration of Hemoglobin in g/dL, to express G-6-PDH activity as U/g of Hemoglobin.

• Wavelength:	340 nm (334-365 nm)
• Pathlength:	1 cm
• Reading:	against air or distil. water
• Temperature:	37°C
• Method:	kinetic
• Reaction:	10+2+5 minutes
• Linearity:	till ΔA/min. 0,060

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• Sample/Reagent: 1/100/200

Let reagent reaches the working temperature before using.

Pipette into a test tube or cuvette labelled: S: Sample:

		S
Working Reagent (*R1+*R2)		1000 µl
Sample		10 µl

Mix kindly and incubate for about 5-10 min. at 37°C. Add:

*Reagent 3		2000 µl

Mix kindly. Exactly 2 min. after, make the FIRST reading of the sample (As1).
Repeat the SECOND readings after 5 min. for the sample (As2).
Determine the absorbance/minute for the Sample (ΔAs/min), as (SECOND readings – FIRST readings) / 5

CALCULATION

Insert the (ΔAs/min) found in the following formula:

$$\text{G6PDH (U/10}^{12}\text{ RBC)} = \Delta\text{As/min. Sample} \times \frac{47778}{N} \times \text{TCF}$$

Where:
N = Red Cell Count divided by 10^{6}
TCF = Temperature Correction Factor (see NOTE 8.)

or

$$\text{G6PDH (U/g Hemoglobin)} = \Delta\text{As/min. Sample} \times \frac{4777,8}{\text{Hb (g/dL)}} \times \text{TCF}$$

Where:
Hb (g/dL) = Hemoglobin conc. determined for each sample
TCF = Temperature Correction Factor (see NOTE 8.)

EXAMPLE
Assay of a sample which had Red Cell Count= 4,5 x 10^{6} mm3, an hemoglobin concentration of 14,2 g/dL and a (ΔAs/min)=0,040 at 37°C.

$$\text{G6PDH (U/10}^{12}\text{ RBC at 37°C)} = 0,040 \times \frac{47778}{4,5} \times 1 = 425$$

$$\text{G6PDH (U/g Hemoglobin)} = 0,040 \times \frac{4777,8}{14,2} \times 1 = 13,46$$

REFERENCE VALUES

G-6-PDH activity at 30°C

(U/10^12 RBC) = 147 - 376
(U/g Hemoglobin) = 4,6 – 13,5

G-6-PDH activity at 37°C

(U/10^12 RBC) = 221 - 570
(U/g Hemoglobin) = 7,0 – 20,5

Values for the newborns may range somewhat higher.

It is suitable that every laboratory determine its reference values.

PERFORMANCE CHARACTERISTICS

These performance characteristics was determined using a spectrophotometer or analyzers typically found in clinical laboratories, under the stated assay conditions.

Linearity: If the absorb./minute is higher than 0,060 use half sample volume and multiply the result x2.

Sensitivity: The minimum detectable is 0,8 U/g Hb or 21,6 U/10^{12} RBC (assuming an Hb conc. of 12,0 g/dL and an RBC count of **4,5 x 10^{6}/mm3).**

Within-run Precision:

	Mean (U/L) ± 2s	CV %
Serum 1	500 ± 24	2,3

Run-to-run (Day-to-day) Precision:

	Mean (U/L) ± 2s	CV %
Serum 1	526 ± 40	3,8

Interferences: See References point 3.

Correlation: A group of 20 sample was assayed by this procedure and using a similar commercially available G6PDH Reagent.
Comparison of the data gave following results:

Linear regression equation y = 1,3975x – 0,6
Correlation coefficient r = 0,9215 n=20

NOTE

1. This assay reflects also the 6-phospogluconate dehydrogenase (6-PGD) activity which generate one molecule of NADPH for one molecule of 6- phosphogluconate formed.
2. Very deep attention must be given to interfering substances: copper and sulfate are strong inhibitors. But other substances are able to influence levels of G6PDH (see References 3.).
3. Reticulocytes have higher G-6-PDH levels than mature Red Cells; it is not recommended to run the assay after a severe hemolytic crisis, since G-6-PDH may appear falsely elevated.
4. A proportional variation of reaction volumes do not modify the result.
5. If the absorb./minute is higher than 0,060 use half sample volume and multiply the result x2.
6. We suggest do not mix Reagents from different Production lots.
7. PAY ATTENTION!
Applications on routine Analyzers may be totally different from what we developed as manual determination, and also from themselves.

8. Temperature Correction Factor

This table permit to change the activity at the different temperature:

My test was made at following temperature ↓		To have values at **37°C,** multiply ↓	To have values at **30°C,** multiply ↓	To have values at **25°C,** multiply ↓
37°C		**x 1**	**x 0,70**	**x 0,51**
30°C		**x 1,43**	**x 1**	**x 0,73**
25°C		**x 1,96**	**x 1,37**	**x 1**

9. If (ΔAs/min) are very, very low, may be possible increase the sample volume (and of course the Control volume).
10. The reagent must be used only for the intended destinations, by expert people and in the due lab. conditions.
11. The clinical diagnosis cannot be done using the result of only one test, but have to be done integrating different lab. and clinical data.

REFERENCES

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