Kinetic Determination of enzyme
Glucose 6-Phosphate Dehydrogenase
in Serum, Plasma and Erythrocytes

4 x 25 ml **REF CY06-100**

PRINCIPLE

The activity of the enzyme Glucose-6-Phosphate Dehydrogenase (E.C. 1.1.1.49) is defined by measuring the speed of the absorbance increase at 340 nm due to the reduction of $NADP^+$ according to the following reaction:

G-6-P + $NADP^+$ $\xrightarrow{\text{G-6-PDH}}$ gluconate-6-P + NADPH + H^+

REAGENTS

Kit components:	REF CY06-100	Quantity
REAGENT 1 Triethyanolamine buffer ph 7.6 EDTA, sodium azide 15 mmol/L	**CY06-100R1**	**4 x 25 ml**
REAGENT 2 (lyo) $NADP^+$	**CY06-100R2**	**1 vial**
REAGENT 3 (lyo) Glucose-6-P	**CY06-100R3**	**1 vial**
***REAGENT 4** Digitonine	**CY06-100R4**	**1 x 20 ml**

STABILITY: store at 2-8°C to keep the reagents stable up to the expiration date on the label.

PREPARATION OF WORKING REAGENTS

REAGENT 2

Dissolve the contents of the vial of Reagent 2 with exactly 3.0 ml of distilled water. Shake gently until complete dissolution.
STABILITY: 4 weeks at 2-8°C.

REAGENT 3

Dissolve the contents of the vial of Reagent 3 with exactly 1.5 ml of distilled water. Shake gently until complete dissolution.
STABILITY: 4 weeks at 2-8°C.

SAMPLE

Serum or plasma: use fresh and absolutely hemolysis free samples.
Erythrocytes (hemolized): follow Procedure 2 to prepare the hemolized.

MANUAL ASSAY PROCEDURE

Wavelength:	Hg 365 nm, 340 nm or Hg 334 nm
Optical path:	1 cm
Reading:	against air
Temperature:	25°C, 30°C, 37°C (serum or plasma) 25°C (erythrocytes)
Reaction time:	3 minutes

1. MEASURE OF G6P-DH IN SERUM OR PLASMA

Let the reagents reach the chosen temperature for the analysis.
Pipette in cuvette:

	25°C – 30°C	37°C
Reagent 1	1.00 ml	1.00 ml
Reagente 2	50 µl	50 µl
Serum/plasma	500 µl	250 µl

Mix, incubate for 10 minutes at the test temperature. Add:

Solution 3	25 µl	25 µl

Mix. Read initial absorbance and start timer simultaneously. Repeat the reading after exactly 1, 2 and 3 minutes. Determine the average value of absorbance change per minute (ΔA/min).

CALCULATION

Calculate the G6P-DH activity (mU/ml) in serum or plasma using the following formulas:

	340 nm	Hg 365 nm	Hg 334 nm
25°C/30°C	500 x ΔA/min	900 x ΔA/min	510 x ΔA/min
37°C	841 x ΔA/min	1514 x ΔA/min	858 x ΔA/min

2. MEASURE OF G6P-DH IN ERYTHROCYTES

Define the number of erythrocytes per ml of blood as normal procedure.

PREPARATION OF SAMPLE

Wash 0.2 ml of blood for three times with 2 ml of saline solution. After each washing, centrifuge for 10 minutes at approximately 3000 rpm/minute.
Re-suspend the washed erythrocytes and centrifuged in 0.5 ml of Reagent 4, incubate for 15 minutes at approximately 4°C, then centrifuge again. To define the activity, use the supernatant. Perform the test within 2 hours of the preparation of the hemolized.

Let the reagents reach the chosen temperature for the analysis.
Pipette in cuvette:

Reagent 1	1.00 ml
Reagent 2	30 µl
Hemolized	15 µl

Mix, incubate for 5 minutes at 25°C. Add:

Reagent 3	15 µl

Mix. Read initial absorbance and start timer simultaneously. Repeat the reading after exactly 1, 2 and 3 minutes. Determine the average value of absorbance change per minute (ΔA/min).

CALCULATION

To calculate the G6P-DH activity in erythrocytes use the following formulas:

	340 nm	Hg 365 nm	Hg 334 nm
mU (erythrocytes in 1 ml of blood*)	33650 x ΔA/min	60571 x ΔA/min	34304 x ΔA/min

* The G6P-DH activity is expressed as mU/10^9 erythrocytes or as mU/g hemoglobin.

To calculate the G6P-DH activity as mU/10^9 erythrocytes, as it is required for a comparison with the normal value, divide the calculated activity (mU in the erythrocytes present in 1 ml of blood) by the number of erythrocytes per ml of blood with erythrocytes count. For example, if you obtain 695 mU for the erythrocytes present in 1 ml of blood, and the result of the erythrocytes count is 5.3 x 10^9 erythrocytes /ml of blood, the G6P-DH activity will result 695 / 5.3 = 131 mU/10^9 erythrocytes.

To calculate the G6P-DH activity as mU/g hemoglobin, use the following formula:

$$\text{G6P-DH (mU/g Hb)} = \frac{\text{mU erythrocytes per ml x 100}}{\text{Hb (g/dl)}}$$

where:
100=factor to convert ml into dl
Hb (g/dl)=hemoglobin concentration for each sample

For example, if you obtain 695 mU for the erythrocytes present in 1 ml of blood, and the determined hemoglobin concentration is 15.0 g/dl, the G6P-DH activity will result (695 x 100) / 15.0 = 4633 mU/g Hb.

REFERENCE VALUES

In serum an activity under normal conditions is not virtually detectable (0-0.18 mU/ml).

In erythrocytes: 118 – 144 mU/10^9 erythrocytes.

Each laboratory should define its own reference values.

PERFORMANCE CHARACTERISTICS

Linearity: up to 50 mU/ml in serum (at 37°C) and 1830 mU in erythrocytes for 1 ml of blood (25°C).
For higher values, dilute 1 volume o f sample with 9 volumes of saline solution, repeat the assay and multiply the result by 10.

Within-run precision:

	Level 1	Level 2
Average (mU/10^9 erythrocytes)	130	560
DS	1.10	8.82
CV %	0.85	1.57

Between-run precision:

	Level 1	Level 2
Average (mU/10^9 erythrocytes)	108	502
DS	1.98	15.5
CV %	1.83	3.09

Correlation: G6P-DH FAR kit shows a correlation coefficient equal to 0.98, in comparison to another kit available on the market.

NOTES

1. (*) dangerous reagent are marked with an asterisk. Refer to safety data sheet.
2. Do not pipette by mouth. Apply normal precautions required to handle laboratory reagents.
3. Chemistry analyzer parameters are available.

DISPOSAL

The product must be used for professional analysis only. The product must be disposed of according to national/international laws.

WARNINGS AND PRECAUTIONS

REAGENT 4: ***H302*** Harmful if swallowed.

REFERENCES

1. A. Kornberg *et al.*, Methods in Enzymology I, Academic Press, New York, p. 323 (1955)

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KEY SYMBOLS

IVD	In Vitro diagnostic medical device
LOT	batch number
	catalog number
REF	temperature limits
	use by
	attenzione
	consult accompanying documents

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