BIOLABO
www.biolabo.fr

MANUFACTURER:
BIOLABO SAS,
Les Hautes Rives
02160, Maizy, France

Lyophilised G6-PDH U.V. Kinetic Method

Reagent for quantitative determination of Glucose-6-phosphate dehydrogenase activity (G6-PDH) [EC 1.1.1.49] in human serum, plasma or erythrocytes

REF 97099	**R1** 20 x 3 mL	**R2** 3 x 1 mL	**R3** 1 x 70 mL	**R4** 1 x 20 mL

TECHNICAL SUPPORT AND ORDERS
Tel: (33) 03 23 25 15 50
Fax: (33) 03 23 256 256

CE

IVD IN VITRO DIAGNOSTIC USE

CLINICAL SIGNIFICANCE (1) (2)

Glucose-6-phosphate dehydrogenase (G6-PDH) is an enzyme of glycolysis pathway in erythrocytes.
Different variants of G6-PDH defined by electrophoretic and kinetic criteria are of high frequency and have clinical consequences in different ethnic groups.
A G6-PDH deficiency may cause haemolytic anemia following ingestion of 8-amino-quinoleine antimalarials, nalidixic acid, nitrofurantoin, phenacetin, large doses of vitamin C and some sulfonamides and sulfones. Deficiency may be the cause of haemolytic disease of newborns in Asians and Mediterraneans.

PRINCIPLE (4) (5)

Reaction scheme (method of Beutler and al.) is as follows:

$$G6\text{-}P + NADP^+ \xrightarrow{\text{G6-PDH}} 6\text{-P-Gluconate} + NADPH + H^+$$

The rate of increase in NADPH concentration measured at 340 nm is proportional to the G-6-PDH activity in the specimen.

REAGENTS

Vial R1 **COENZYME** Freeze-dried

$NADP^+$	310	mmo/L

Vial R2 **SUBSTRATE** Freeze-dried

Glucose-6-phosphate	0.6	mmol/L

Vial R3 **BUFFER**

Tris Buffer pH 8.0	100	mmol/L
$MgCl_2$	10	mmol/L
EDTA	0.5	mmol/L
Preservative		

flacon R4 **HEMOLYSING SOLUTION**

Digitonin	0.2	g/L

SAFETY CAUTIONS

BIOLABO reagents are designated for professional, in vitro diagnostic use.

- Use adequate protections (overall, gloves, glasses).
- Do not pipette by mouth.
- In case of contact with skin or eyes, thoroughly wash affected areas with plenty of water and seek medical advice.
- Reagents contain sodium azide (concentration < 0.1%) which may react with copper and lead plumbing. Flush with plenty of water when disposing.
- Material Safety Data Sheet is available upon request.
- Waste disposal: Respect legislation in force in the country.

All specimens should be handled as potentially infectious, in accordance with good laboratory practices using appropriate precautions. Respect legislation in force in the country.

REAGENTS PREPARATION

Add promptly 3 mL of buffer (vial R3) to the contents of vial R1 (Coenzyme) and 1 mL of buffer (vial R3) to the contents of vial R2 (Substrate).

Mix gently and wait for complete dissolution before using reagents (approximately 2 minutes).

Hemolysing solution (vial R4) is ready for use.

STABILITY AND STORAGE

Store at 2-8°C, well recapped in the original vial and away from light.

- Stored and used as described in the insert, unopened reagents are stable until expiry date stated on the label.
- After reconstitution working reagents (vial R1 and R2) are stable at least for 6 months when free from contamination.
- Don't use working reagents after expiry date stated on the label. Discard any reagent if cloudy.

SPECIMEN COLLECTION AND HANDLING

Unhemolysed serum or plasma (heparinised or EDTA). Avoid oxalate and fluoride.

Erythrocytes: Use an hemolysate of whole blood prepared as follows:

(Detailed Procedure available upon request)

1. Determine the concentration of haemoglobin (Hb in g/dL).
2. Wash 3 times 0,2 mL of homogenised blood with 2 mL of saline solution 0.9 g/dL. Centrifuge between each washing and eliminate the supernatant (avoiding elimination of erythrocytes).
3. After the last washing, suspend the washed erythrocytes in 0,9 mL of haemolysing solution (vial R4)
4. Let stand for 15 minutes at 2-8°C and centrifuge again. Use the supernatant (hemolysate) within 1 hour.

INTERFERENCES (2) (3)

This assay reflects also the 6-Phosphogluconate dehydrogenase (6-PGD) activity which generates one molecule of NADPH for one molecule of 6-Phosphogluconate formed.

G6PDH (UI/L) at 37°C in the specimen	Interfering	Results
169 UI/L	Ascorbic acid	Do not interfere up to 25 mg/dL
1756,7 UI/L	Turbidity	Do not interfere up to 0,2 %
1846,7 UI/L	Glucose	Do not interfere up to 1100 mg/dL
2003,3 UI/L	Bilirubin	Negative interference above 7,5 mg/dL

For a more comprehensive review of factors affecting this assay refer to the publication of Young D.S.

MATERIAL REQUIRED BUT NOT PROVIDED

1. Basic medical analysis laboratory equipment.
2. Normal and pathological control sera or hemolysatel

Manufacturer | Use by | IVD In vitro diagnostic | Temperature limitation | REF Catalogue number | See insert | LOT Batch number | Store away from light | Σ sufficient for | → dilute with

CALIBRATION

Results will depend on the accuracy of the instrument calibration, the time counting, the respect of reagent/specimen ratio and the temperature control.
It is recommended to use the theoretical calibration factor (§ **CALCULATION**) or a seric calibrator traceable to a reference method or a reference hemolysate.

QUALITY CONTROL

- REF 95089 Normal Control G6-PDH
- REF 95289 Deficient Control G6-PDH
- Any assayed control sera or a whole blood specimen referring to the same method.
- External quality control program.

It is recommended to control in the following cases:

- At least once a run.
- At least once within 24 hours.
- When changing vial of reagent.
- After maintenance operations on the instrument.

If control is out of range, apply following actions:

1. Repeat the test with the same control.
2. If control is still out of range, prepare a fresh control and repeat the test.
3. If control is still out of range, verify analysis parameters: Wavelength, temperature, specimen/reagent ratio, time counting, calibration factor.
4. If control is still out of range, use a new vial of reagent and reassay
5. If control is still out of range, please contact BIOLABO technical support or your local Agent.

EXPECTED VALUES (2)

In Erythrocytes at 37°C

Conventional Units	International Units
IU/g of Hb: 12.1 ± 2.09	MIU/molHb: [0.78 ± 0.13]
IU/10^{12} Erythrocytes: 351 ± 60.6	nIU/Erythrocytes: [0.35 ± 0.06]
IU/mL of Erythrocytes: 4.11 ± 0.71	KIU/L of Erythrocytes: [4.11 ± 0.71]

Each laboratory should establish its own normal ranges for the population that it serves..

In Serum at 37°C

Normal value: No detectable G6-PDH activity.

PERFORMANCE CHARACTERISTICS

Studies performed using hemolyzate as specimen (Cobas Mira).

Within run n=20	*Low level*	*Normal level*	*High level*
Mean(IU/L)	478	925	2280
S.D. (IU/L)	13.3	33.2	31.2
C.V. %	2.8	3.6	1.4
Critères CV%	< 4.5%	< 4.5%	< 3.8%

Between run	*Low level (n=15)*	*Normal level (n=15)*	*High level (n=30)*
Mean (IU/L)	276	535	918
C.V. %	5.8	4.8	3.9
S.D(IU/L)	16.0	25.5	35.5
C.V. %	5,8	4,8	3,9
Critères CV%	< 6%	< 6%	< 5%

Detection limits: approximately 21 IU/L.

Sensitivity: approx. 0.020 ΔAbs/min /1000 $IU.L^{-1}$ of blood

Comparison study with commercially available reagent (UV Kinetic method):

102 hemolysates within 110 and 1500 IU/L have been evaluated with both reagents on Cobas Mira:

y = 0.9992x – 6.5933 r= 0.9874

LINEARITY

Above 4000 UI/L (0.080 ΔAbs/mn), dilute specimen (serum, plasma, hemolysate) with saline solution 0.9 g/dL and reassay taking into account dilution factor to calculate the result. Linearity limits depend on the specimen/reagent ratio.

MANUAL PROCEDURE

Let stand reagents and specimens at room temperature.

Pipette into thermostated cuvette at 37°C:	**Serum Assay**	**Hemolysate Assay(1)**
Reagent R1	2 mL	3 mL
Serum	1 mL	
Hemolysate		50 µl
Mix and incubate for 5 minutes at 37°C (30°C, 25°C)		
Reagent R2	100 µl	100 µl
Mix and record initial absorbance at 340 nm after 30 seconds. Record the absorbance again every minutes during 3 minutes. Calculate absorbance change per minute (ΔAbs/min). If activity is lower, timing of measurement may be prolonged.		

Notes (1):
1-Preparation of the hemolysate: see § **SPECIMEN COLLECTION AND HANDLING**.
2- Hemolysate assay may be realised directly in the vial R1

CALCULATION (2)

Calculate G6-PDH activity as follows:

Serum: IU/L = (ΔAbs/min) x 492

Erythrocytes: IU/L of blood = (ΔAbs/min) x 50 000

Result in units per gramme of Hemoglobin

$$\textbf{IU/g Hb} = \frac{\textbf{(ΔAbs/min x 5 000)}}{\textbf{Hb expressed in g/dL}}$$

Example: if ΔAbs/min = 0.030 and Hb = 14.5 g/dL

$$\text{IU/g Hb} = \frac{0.030 \times 5000}{14.5} = 10.3$$

Result in units per 10^{12} Erythrocytes (# Result in IU/g Hb x 29)

$$\textbf{IU/}10^{12}\textbf{ Erythrocytes} = \frac{\textbf{ΔAbs/min x 50 000}}{\textbf{Number of erythrocytes in }10^{12}\textbf{/L}}$$

Example: if ΔAbs/min = 0.030 and erythrocytes number = 4.2. 10^{12}/L

$$\text{IU/}10^{12}\text{ erythrocytes} = \frac{0.030 \times 50\,000}{4.2} = 357$$

Result in units per mL of Erythrocytes (# Result in IU/g Hb x 0.34)

$$\textbf{IU/ml of Erythrocytes} = \frac{\textbf{(ΔAbs/min x 5 000)}}{\textbf{Hematocrit (\%)}}$$

REFERENCES

(1) TIETZ N.W. Textbook of clinical chemistry, 3rd Ed. C.A. Burtis, E.R. Ashwood, W.B. Saunders (1999) p. 1645-1650.
(2) Clinical Guide to Laboratory Test, 4th Ed., N.W. TIETZ (2006) p. 457-458.
(3) YOUNG D.S., Effect of Drugs on Clinical laboratory Tests, 4th Ed. (1995) p. 3-294
(4) BEUTLER et al., International comittee for standardisation in Haematology: « Recommended Methods for Red Cell Enzyme Analysis » British Journal of Haematology, (1977), 35, p.331-340.
(5) BEUTLER E., Red cell metabolism,: A manual of biochemical methods (3rd Ed.) Orlando, Grune et Stratton (1984), p.68-70