BIOLABO
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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 97089	**R1** 2 x 30 mL	**R2** 1 x 3 mL	**R3** 1 x 20 mL

TECHNICAL SUPPORT AND ORDERS
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CE

IVD

Made In France

I: corresponds to significant modifications

I INTENDED USE

This reagent is designated for professional use in laboratory (manual or automated method).
It allows the quantification of Glucose-6-phosphate dehydrogenase (G6-PDH) to evaluate its activity in human serum, plasma or erythrocytes.

GENERALITIES (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:

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

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

I REAGENTS

R1	**G6-PDH**	Coenzyme-Buffer
$NADP^{+}$		310 mmol/L

DANGER :
Before reconstitution :
Acute Tox. 2: H300 - Fatal if swallowed. Acute Tox. 4: H332 - Harmful if inhaled. Aquatic Chronic 2: H411 - Toxic to aquatic life with long lasting effects.
Substances that contribute to the classification sodium azide 2,5- < 10%; EDTA Na2 1,0- < 2.5%

R2	**G6-PDH**	Substrate
Glucose 6 phosphate		0,6 mmol/L

CAUTION :
Before reconstitution,
Acute Tox. 4: H302 - Nocif en cas d´ingestion. Aquatic Chronic 3: H412 - Nocif pour les organismes aquatiques, entraîne des effets néfastes à long terme. Substances qui contribuent à la classification azide de sodium 1-< 2,5%

Vial R1 and R2 :
P280: Wear protective gloves/protective clothing/respiratory protection/eye protection/protective footwear. P301+P310: IF SWALLOWED: Immediately call a POISON CENTER/doctor. P330: Rinse mouth. P501: Dispose of contents/container in accordance with regulations on hazardous waste or packaging and packaging waste respectively.

Once reconstituted, reagents R1 and R2 are not classified as dangerous according to CLP regulation 1272/2008 (CE)

R3	**G6-PDH**	Hemolysing solution
Digitonine		0,2 g/L

According to CLP regulation 1272/2008 (CE), this reagent is not classified as dangerous.

SAFETY CAUTIONS

- Refer to current Material Safety Data Sheet available on request or on www.biolabo.fr
- Verify the integrity of the contents before use.
- Waste disposal: Respect legislation in force in the country.
- All specimens or reagents of biological origin should be handled as potentially infectious. Respect legislation in force in the country.

I Any serious incident that has occurred in connection with the device is notified to the manufacturer and the competent authority of the Member State in which the user and/or patient is based.

REAGENTS PREPARATION

Reagent 1: Add promptly 30 mL of demineralized water into vial R1
Reagent 2 : Add promptly 3 mL of demineralized water into vial R2
Mix gently and wait for complete dissolution.
Hemolysing solution (vial R3) is ready for use.

STABILITY AND STORAGE

Stored away from light, well caped in the original vial at 2-8°C, when stored and used as described, reagents are stable:

Unopened:

- Until expiry date stated on the label of the kit.

Once opened:

- After reconstitution working reagents (R1 and R2) are stable at least for 1 month 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:

Prepare an hemolysate of whole blood prepared as follows:

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.

LIMITES (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.
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. Spectrophotometer (340nm, 37°C)
3. Saline solution (9 g/L)

QUALITY CONTROL

- REF 95089 Normal Control G6-PDH
- REF 95289 Deficient Control G6-PDH
- 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. Prepare a fresh control serum and repeat the test.
2. If control is still out of range, use a new vial of calibrator or a fresh calibrator and repeat the test.
3. If control is still out of range, repeat the tests with a new vial of reagent.

If control is still out of range, please contact BIOLABO technical support or your local Agent.

REFERENCE INTERVALS (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.

PERFORMANCES

On spectrophotometer, 340 nm, 37°C:

Specimen: hemolysate

Detection limit: approximately 26 IU/L of blood.

Linearity range: from 35 IU/L (LOQ) to 4000 IU/L.

Above, dilute specimen (hemolysate) with saline solution 0.9 g/dL and re-assay taking into account dilution factor to calculate the result.
Linearity limits depend on the specimen/reagent ratio.

Precision:

Within run N = 18	Low level	High level
Mean (IU/L)	264	537
S.D. (IU/L)	13.6	23.7
C.V. %	5.2	4.4

Between run N = 15	Low level	High level
Mean (IU/L)	276	535
S.D. (IU/L)	16.0	25.5
C.V. %	5.8	4.8

Analytical sensitivity : 0.020 ΔAbs /1000 UI.L-1 de sang

Comparison study with commercially available reagent (UV method) :

y = 1, 0271 + 13,1 r=0,9933

Interferences:

Turbidity	No interference up to 0.281 abs
Ascorbic acid	No interference up to 2500 mg/dL
Total bilirubin	Positive interference from 90 µmol/L
Glucose	No interference up to 1110 mg/dL

Other substances may interfere (see § Limits)

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 calibrator traceable to a reference method or a reference hemolysate.

PROCEDURE

Manual Procedure:

Let stand reagents and specimens at room temperature.

Prepare hemolysate (Refer § Specimen collection and Handling)

Pipette into thermostatic reading 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		
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.		

CALCULATION (2)

Serum: IU/L = (ΔAbs/min) x 492

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

Result in units per gramme of Hemoglobin

$$\text{IU/g Hb} = \frac{(\Delta\text{Abs/min} \times 5\,000)}{\text{Hb expressed in g/dL}}$$

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)

$$\text{IU/}10^{12}\text{ Erythrocytes} = \frac{\Delta\text{Abs/min} \times 50\,000}{\text{Number of erythrocytes in } 10^{12}\text{/L}}$$

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)

$$\text{IU/ml of Erythrocytes} = \frac{(\Delta\text{Abs/min} \times 5\,000)}{\text{Hematocrit (\%)}}$$

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*