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

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CE

IVD **IN VITRO DIAGNOSTIC USE**

CLINICAL SIGNIFICANCE (1) (2)

Glucose-6-phosphate dehydrogenase (G-6-PDH) is an enzyme of glycolysis pathway in erythrocytes.
Different variants of G-6-PDH defined by electrophoretic and kinetic criteria are of high frequency and have clinical consequences in different ethnic groups.
A G-6-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:

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

The rate of increase in NADPH concentration measured at 340 nm is proportional to the G6-PDH activity in the specimen.

REAGENTS

Vial R1 **COENZYME-BUFFER**

Tris Buffer pH 8.0	100	mmol/L
$MgCl_2$	10	mmol/L
EDTA	0.5	mmol/L
$NADP^+$	310	mmo/L
Preservative		

Vial R2 **SUBSTRATE**

Glucose-6-phosphate	0.6	mmol/L

Vial R1: Before reconstitution:: Xi, R36/37/38
Irritating to eyes, respiratory system and skin.

Vials R1 / R2: Before reconstitution: T, R28-32-50/53
Very toxic if swallowed. Contact with acids liberates toxic gas. Very toxic for aquatic organisms, may cause long-term adverse effects in the aquatic environment.
S22-26-28,-38: Do not breathe dust. In case of contact with eyes rinse immediately with plenty of water and seek medical advice. After contact with skin wash immediately with plenty of water. In case of insufficient ventilation wear suitable respiratory equipment

Vials R1 / R2: Once reconstituted: None

flacon R3 **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 30 mL of distilled or deionised water to the contents of vial R1 (Coenzyme-Buffer).
Add promptly 3 mL of distilled or deionised water to the contents of vial R2 (Substrate).
Mix gently and wait for complete dissolution before using reagents (approximately 2 minutes).
Haemolysing solution (vial R3) 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.
- Working reagents (vial 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. 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 R3)
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.
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 hemolysate.

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 Contrôle Normal G6-PDH
- REF 95289 Contrôle Déficient 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.

Within run N = 18	Low level	High level
Mean U/10^{12} Erythrocytes	264	537
S.D. U/10^{12} Erythrocytes	13.6	23.7
C.V. %	5.2	4.4

Between run N = 15	Low level	High level
Mean U/10^{12} Erythrocytes	276	535
S.D. U/10^{12} Erythrocytes	16.0	25.5
C.V. %	5.8	4.8

Detection limits: approximately 31 IU/10^{12} Erythrocytes.

Comparison study with commercially available reagent:

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

LINEARITY

If Δ Abs/min > 0.060, 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 (30°C, 25°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 secondes. Record the absorbance again every minute during 3 minutes. Calculate absorbance change per minute (ΔAbs/min). If activity is lower, timing of measurement may be prolonged.		

Notes:

1-Preparation of the hemolysate: see § **SPECIMEN COLLECTION AND HANDLING**

CALCULATION (2)

Calculate G6-PDH activity as follows:

Serum: IU/L = (ΔAbs/min) x 492

Erythrocytes:

UI/L of blood = (ΔAbs/min) x 50 000

Result in units per gramme of Hemoglobin

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

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)

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

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

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

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

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

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. 458-457.*

(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*


Manufacturer


Use by


In vitro diagnostic


Temperature limitation


Catalogue number


See insert


Batch number


Store away from light


sufficient for

dilute with