CHRONOLAB

CE
IVD

PHI

G6P. Kinetic UV

Quantitative determination of phosphohexose isomerase (PHI)

PACKAGING

Ref.: 101-0742	Cont.: 20 x 3 mL

Store at 2-8º C

CLINICAL SIGNIFICANCE

The phosphohexose isomerasa (PHI) is used as an index of metastasis in patients with breast or prostate carcinoma and to control the treatment's success. It is a low specificity oncologic indicator[4,5].

Clinical diagnosis should not be made on a single test result; it should integrate clinical and other laboratory data.

PRINCIPLE OF THE METHOD

The phosphohexose isomerasa (PHI) catalyzes the conversion of fructose-6-phosphate (F6P) in glucose-6-phosphate (G6P), according the following reaction:

$$\text{Fructose-6-phosphate (F6P)} \xrightarrow{\text{PHI}} \text{Glucose-6-phosphate (G6P)}$$

$$\text{G6P+ NAD+} \xrightarrow{\text{G6P-DH}} \text{6-Phosphogluconate + NADH + H}^+$$

The rate of NADPH formation, measured photometrically, is proportional to the catalytic concentration of PHI present in the sample[1].

REAGENTS

R 1 Buffer	TRIS pH 8.5	100 mmol/L
R 2 Substrate	Fructose-6-phosphate (F6P)	3 mmol/L
	NAD	0.8 mmol/L
	Glucose-6-phosphate-dehidrogenase	1500 U/L

Optional (not included in the kit)

Contro-N	Ref.: 101-0252	4 x 5 mL	Lyophilized human control serum
	Ref.: 101-0083	20 x 5 mL	
Contro-P	Ref.: 101-0253	4 x 5 mL	Lyophilized human control serum
	Ref.: 101-0084	20 x 5 mL	

PREPARATION

Working reagent (WR):

Dissolve (→) one tablet of R 2 Substrate with one vial of R 1 Buffer.

Cap vial and mix gently to dissolve contents.

Stability: 30 hours at 2-8º C.

STORAGE AND STABILITY

All the components of the kit are stable until the expiration date on the label when stored tightly closed at 2-8º C, protected from light and contaminations prevented during their use.

Do not use the tablets if appears broken.

Do not use reagents over the expiration date.

Signs of reagent deterioration:

- Presence of particles and turbidity.
- Blank absorbance (A) at 340 nm ≥ 0.70.

ADDITIONAL EQUIPMENT

- Spectrophotometer or colorimeter measuring at 340 nm.
- Thermostatic bath at 25º C, 30º C ó 37º C (± 0.1º C)
- Matched cuvettes 1.0 cm light path.
- General laboratory equipment.

SAMPLES

Serum or plasma[1]. Stability: 7 days at 2-8º C.

PROCEDURE

Notes: CHRONOLAB SYSTEMS has instruction sheets for several automatic analyzers. Instructions for many of them are available on request.

1. Assay conditions:
   Wavelength: . . . . . 340 nm
   Cuvette: . . . . . 1 cm light path
   Constant temperature . . . . . 25º C /30º C / 37º C
2. Adjust the instrument to zero with distilled water or air.
3. Pipette into a cuvette:

WR (mL)	1.5
Sample (µL)	100

4. Mix, incubate for 3 minutes (30º C or 37º C) or 5 minutes (25º C)..
5. Read initial absorbance (A) of the sample, start the stopwatch and read absorbances at 1 minute intervals thereafter for 3 minutes.
6. Calculate the difference between absorbances and the average absorbance differences per minute (ΔA/min).

CALCULATIONS

ΔA/min x 2540* = U/L of PHI

Units: One international unit (IU) is the amount of enzyme that transforms 1 µmol of substrate per minute, in standard conditions. The concentration is expressed in units per litre of sample (U/L).

Temperature conversion factors

To correct results to other temperatures multiply by:

Assay temperature	Conversion factor to		
	25º C	30º C	37º C
25º C	1.00	1.27	1.47
30º C	0.79	1.00	1.16
37º C	0.68	0.86	1.00

QUALITY CONTROL

Control sera are recommended to monitor the performance of assay procedures.

If control values are found outside the defined range, check the instrument, reagents and technique for problems.

Each laboratory should establish its own Quality Control scheme and corrective actions if controls do not meet the acceptable tolerances.

REFERENCE VALUES[1]

	25º C	30º C	37º C
Up to	75 U/L	95 U/L	110 U/L

These values are for orientation purpose; each laboratory should establish its own reference range.

PERFORMANCE CHARACTERISTICS

Measuring range: Up to linearity limit of 0.090 ΔA/min.

If the results obtained were greater than linearity limit, dilute the sample 1/5 with NaCl (9 g/L) and multiply the result by 5.

Accuracy: Results obtained using CHRONOLAB reagents (y) did not show systematic differences when compared with other commercial reagents (x).

The results of the performance characteristics depend on the analyzer used.

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INTERFERENCES

Hemolysis interferes with the assay.

A list of drugs and other interfering substances with PHI determination has been reported by Young et. [al2,3].

BIBLIOGRAPHY

1. Bueding E. Mackinnon J. Biol. Chem. 215- 507,513 (1955)
2. Young DS. Effects of drugs on Clinical Lab. Tests, 4th ed AACC Press, 1995.
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