Reactivos GPL

Barcelona, España

CE

- ACP -

ACID PHOSPHATASE

α-Naphtylphosphate. Kinetic.

Store at: +2+8ºC.

Presentation:
Cod. EZ001 CONT: R1 1 x 45 mL. + R2 19 Comp. x → 2 mL.

Procedure

Quantitative determination of acid phosphatase (ACP).

Only for *in vitro use in clinical laboratory* (IVD)

TEST SUMMARY

Acid phosphatase (ACP) catalyses the hydrolysis of α-naftyl phosphate at pH 5.2, liberating α-naphtol. The α-naphtol formed reacts with a diazonium salt (Fast Red TR) according to the following reactions:

$$\alpha\text{-Naftyl phosphate} + H_2O \xrightarrow{ACP} \alpha\text{-Naphtol+ phosphate}$$

$$\alpha\text{-Naphtol} + \text{Fast Red TR} \longrightarrow \text{Azoic chromogen}$$

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

REAGENTS COMPOSITION

R 1 Buffer	Sodium citrate pH 5.2	50 mmol/L
R 2 Substrate	α-Naftyl phosphate Fast Red TR	10 mmol/L 6 mmol/L
R 3 Tartrate	Sodium tartrate	2 mmol/L
R 4 (not included)	Acetic acid	0.5 mol/L

REAGENT PREPARATION AND STABILITY

Working reagent (WR): Dissolve (→) 1 tablet of R.2 in 2 mL. of R.1.

Cap and mix gently to dissolve contents.
Stability: 2 days at 2-8ºC or 6 hours at room temperature.
R.3: Ready to use.
All the components of the kit are stable until the expiration date on the label when stored at 2-8ºC, protected from light and contamination prevented during their use.
Do not use reagents over the expiration date.
Do not use tablets if appears broken.

Signs of Reagent deterioration:

- Presence of particles and turbidity.
- Blank absorbance (A) at 405 nm. ≥ 0.44

All the reagents of the kit are stable up to the end of the indicated month and year of expiry. Store tightly closed at 2-8º C. Do not use reagents over the expiration date.

SPECIMEN

Serum[1]. Use only clear and unhemolyzed serum, separated from the clot as soon as possible. Do not use plasma.
Acid phosphatase is very labile; stabilize by adding 50 µL of acetic acid (R.4) per mL of the sample. Stability: 7 days at 2-8ºC.

MATERIAL REQUIRED BUT NOT PROVIDED

- Spectrophotometer or colorimeter measuring at 405 nm.
- Thermostatic bath at 30ºC o 37ºC (± 0.1ºC)
- Matched cuvettes 1.0 cm light path.

General laboratory equipment.

TEST PROCEDURE

1. Assay Conditions
   - Wavelength : ..... 405 nm.
   - Cuvette: ..... 1 cm light path.
   - Constant temperature . . . . .30ºC / 37ºC.
2. Adjust the instrument to zero with distilled water or air.
3. Pipette into a Cuvette(note 1):

	ACP Total	ACP Non Prostatic
WR (mL)	1.0	1.0
R 3 (µL.)	--	10
Sample (µL.)	100	100

4. Mix and incubate for 5 minutes.
5. Read the absorbance (A) of the sample, start the stopwatch and read absorbance at 1 min. interval thereafter for 3 min.
6. Calculate the difference of absorbance and the average absorbance difference per minute (ΔA/min.)

CALCULATIONS(Note 2)

ΔA/min x 750* = U/L of ACP Total

750* x (ΔA/min ACP (Total) - (ΔA/min ACP Non inhibutor by Tartrate) x = U/L de ACP Prostátic.

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).

QUALITY CONTROL

Control sera are recommended to monitor the performance of the procedure, Normal and Pathological. If control values are found outside the defined range, check the instrument, reagents and calibrator for problems.

Serum controls are recommended for internal quality control. Each laboratory should establish its own Quality Control scheme and corrective actions.

REFERENCE VALUES[1]

Total Acid Phosphatase	30ºC	37ºC
Men:	< 4,3 U/L	< 5,4 U/L.
Women:	< 3,1 U/L	< 4,2 U/L.
Prostatic acid phosphatase :	< 1,5 U/L	< 1,7 U/L.

(These values are for orientation purpose).

It is suggested that each laboratory establish its own reference range.

CLINICAL SIGNIFICANCE

Acid phosphatase is an enzyme present in almost all weaves of the organism, being particularly high in prostate, stomach, liver, muscle, spleen, erythrocytes and platelets.
High levels of acid phosphatase are found in prostatic phatologies as hypertrophy, prostatitis or carcinoma. In hematological disorders, bones or liver diseases as well as in Paget's or Gaucher`s diseases.
Decreased serum acid phosphatase has no clinical significance[1,4,5].
Clinical diagnosis should not be made on a single test result; it should integrate clinical and other laboratory data.

REAGENT PERFORMANCE

- *Measuring Range*:
  From detection limit of 0.13 U/L. to linearity limit of 150 U/L., under the described assay conditions.
  If results obtained were greater than linearity limit, dilute the sample 1/2 with NaCl 9 g/L. and multiply result by 2.
- *Precision*:

	Intra-assay n= 20		Inter-assay n= 20	
Mean (U/L)	23.67	2.56	23.6	2.6
SD	0.22	0.07	0.22	0.07
CV (%)	0.95	2.90	0.92	2.76

- Sensitivity: 1 U/L = 0.0034 ΔA/min
- Accuracy: Results obtained GPL reagents did not show systematic differences when compared with other commercial reagents.
  The results obtained using 50 samples were the following:
  Correlation coefficient (r): 0.99
  Regression Equation: y=0.9977x + 1.486

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

INTERFERING SUBSTANCES

- Hemolysis interferes due the high concentration of acid phosphatase in red cells[1]
- A list of drugs and other interfering substances with ACP determination has been reported by Young et. al[2,3].

NOTAS

1. Usar puntas de pipeta desechables limpias para su dispensación.
2. Formulación para obtener la constante::.

ΔA/min x 750 = U/L de ACP

$$* \frac{Tv \times 1000}{\varepsilon \times LP \times Sv}$$

Tv= Total volume in mL
ε diazo dye = 12.9 at 405 nm
LP= Light path
Sv= Sample volume in mL

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