Reactivos GPL

Barcelona, España

CE

- ALP LQ -

ALKALINE PHOSPHATASE

p-Nitrophenilphosphate. Kinetic. Liquid.DGCK

Store at: +2+8ºC.

Presentation:
Cod. EZ002LQ CONT: R1 1 x 100 + R2 1 x 25 mL .
EZ003LQ CONT: R1 2 x 100 + R2 2 x 25 mL

Procedure

Quantitative determination of alkaline phosphatase (ALP).

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

TEST SUMMARY

Alkaline phosphatase (ALP) catalyses the hydrolysis of p-nitrophenyl phosphate at pH 10.4, liberating p-nitrophenol and phosphate, according to the following reaction:

$$\text{p-Nitrophenylphosphate} + H_2O \xrightarrow{\text{ALP}} \text{p-Nitrophenol} + \text{Phosphate}$$

The rate of p-Nitrophenol formation, measured photometrically, is proportional to the catalytic concentration of alkaline phosphatase present in the sample[1,2]

REAGENTS COMPOSITION

R 1 Buffer	Diethanolamine (DEA) pH 10.4 Magnesium chloride	1 mmol/L 0.5 mmol/L
R 2 Substrate	p-Nitrophenylphosphate (*p*NPP)	10 mmol/L

REAGENT PREPARATION AND STABILITY

Working reagent (WR):
Mix 1 volume of R2 with 4 volumes of R1.
Stability: 30 days at 2-8ºC or 10 days at room temperature (15-25ºC).
Signs of Reagent deterioration:
- Presence of particles and turbidity.
- Blank absorbance (A) at 405 nm. ≥ 1.30

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.

SPECIMEN

Serum or heparinzed plasma [1].
Use unhemolyzed serum, separated from the clot as soon as possible. Stability: 3 days at 2-8º C.

MATERIAL REQUIRED BUT NOT PROVIDED

- Spectrophotometer or colorimeter measuring at 405 nm.
- Thermostatic bath at 25ºC, 30ºC or 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 . . . . .25ºC / 30ºC / 37ºC.
2. Adjust the instrument to zero with distilled water or air.
3. Pipette into a Cuvette:

WR (mL)	1.2
Sample (µL.)	20

4. Mix and incubate for 1 minute.
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 3300* = U/L de ALP

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		
	25ºC	30ºC	37ºC
25ºC	1.00	1.22	1.64
30ºC	0.82	1.00	1.33
37ºC	0.61	0.75	1.00

QUALITY CONTROL

Control sera are recommended to monitor the performance of the procedure, Control H Normal Ref. QC003 and Control H Pathological Ref. QC004. 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]

	25ºC	30ºC	37ºC
Children (1-14 years) up to	<400 U/L	<480 U/L	<645 U/L
Adults up to	60 - 170 U/L	73 - 207 U/L	98 - 279 U/L

Factors affecting ALP activities in a normal population include exercise, periods of repaid growth in children and pregnancy.
(These values are for orientation purpose).

It is suggested that each laboratory establish its own reference range

CLINICAL SIGNIFICANCE

Alkaline phosphatase is an enzyme present in almost all weaves of the organism, being particularly high in bone, liver, placenta, intestine and kidney.
Both increases and decreases of plasma ALP are of importance clinically.
Causes of increased plasma ALP: Paget's disease of bone, obstructive liver disease, hepatitis, hepatotoxicity caused by drugs or osteomalacia.
Causes of decreased plasma ALP: Cretinism and vitamin C deficiency[1,5,6].
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 0.6845 U/L. to linearity limit of 1200U/L., under the described assay conditions.
  If results obtained were greater than linearity limit, dilute the sample 1/10 with NaCl 9 g/L. and multiply result by 10.
- *Precision*:

	Intra-assay n= 20		Inter-assay n= 20	
Mean (U/L)	174	443	175	434
SD	0.72	1.56	3.93	11.93
CV	0.41	0.35	3.93	2.75

- Sensitivity: 1 U/L = 0.0003 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 $(r)^2$: 0.99938
  Regression equation: y=1.025x – 1.105
  The results of the performance characteristics depend on the analyzer used.

INTERFERING SUBSTANCES

- Fluoride, oxalate, citrate and EDTA inhibit alkaline phosphate activity and should therefore not be used as anticoagulants. Haemolyses interferes due to the high concentration of alkaline Phosphatase in red cells[1,2]
- A list of drugs and other interfering substances with ALP determination has been reported by Young et. al [3, 4].

NOTES

1. Use clean disposable pipette tips for its dispensation.
2. Formulation to reach constant:

ΔA/min. x 3300* = U/L ALP

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

Tv= Total volume in mL
ε p-Nitrophenol = 18.45 at 405 nm
LP= Light path
Sv= Sample volume in mL

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