Quantitative determination of alkaline phosphatase (ALP) IVD

Store at 2-8°C

**PRINCIPLE OF THE METHOD**

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

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\text{p-Nitrophenylphosphate} + \text{H}_2\text{O} \rightarrow \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.

**CLINICAL SIGNIFICANCE**

Alkaline phosphatase is an enzyme present in almost all weaves of the organism, being particularly high in bone, liver, placenta and intestine and kidney.

Both increases and decreases of plasma ALP are of importance clinically. Causes of decreased plasma ALP: Cretinism and vitamin C deficiency, osteomalacia. Causes of increased plasma ALP: Paget's disease of bone, obstructive liver disease, hepatitis, hepatotoxicity caused by drugs or osteomalacia.

Both increases and decreases of plasma ALP are of importance clinically. Clinical diagnosis should not be made on a single test result; it should integrate clinical and other laboratory data.

**REAGENTS**

R 1

- Diethanolamine (DEA) pH 10.4 1 mmol/L
- Magnesium chloride 0.5 mmol/L

R 2

- p-Nitrophenylphosphate (pNPP) 10 mmol/L

**PREPARATION**

Working reagent (WR):

- Mix: 4 vol. (R1) Buffer + 1 vol. (R2) Substrate

Stability: 21 days at 2-8°C or 10 days at room temperature (15-25°C).

**STORAGE AND STABILITY**

All the components of the kit are stable until the expiration date on the label when stored tightly closed at 2-8°C, protected from light and contaminations prevented during their use. Do not use reagents after the expiration date.

**ADDITIONAL EQUIPMENT**

- Spectrophotometer or colorimeter measuring at 405 nm.
- Thermostatic bath at 25°C, 30°C or 37°C (± 0.1°C).
- Matched cuvettes 1.0 cm light path.
- General laboratory equipment.

**SAMPLES**

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

**PROCEDURE**

1. **Assay conditions:**
   - Wavelength: 405 nm
   - Wavenumber: 2.00
   - Blank absorbance: 0.000
   - Constant temperature: 25°C / 30°C / 37°C
   - End absorbance: 0.000
   - Blank absorbance: 0.000

2. Mix, incubate for 1 minute.

3. Read initial absorbance (A) of the sample, start the stopwatch and read absorbances at 1 minute intervals thereafter for 3 minutes.

4. Calculate the difference between absorbances and the average absorbance differences per minute (ΔA/min).

**CALCULATIONS**

\[
\text{ΔA/min x } 3300 = \text{U/L de ALP} \]

**QUALITY CONTROL**

Control sera are recommended to monitor the performance of assay procedures: TYB SERIES Biochemistry Control Normal and Pathologic (Ref. CTN 29766 and CTP 29866).

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**

- **Children (1-14 years)**: 400 U/L < 480 U/L < 645 U/L
- **Adults**:
  - 60 - 170 U/L
  - 73 - 207 U/L
  - 98 - 279 U/L

Factors affecting ALP activities in a normal population include exercise, periods of rapid growth in children and pregnancy.

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

**PERFORMANCE CHARACTERISTICS**

Measuring range: From detection limit of 0.6845 U/L to linearity limit of 1200 U/L.

If the results obtained were greater than linearity limit, dilute the sample 1/2 with NaCl 9 g/L and multiply the result by 2.

**Precision**

- **Intra-assay (n=20)**
  - Mean (U/L): 174 ± 443
  - SD: 0.72 ± 1.5
  - CV (%): 0.41 ± 0.35
- **Inter-assay (n=20)**
  - Mean (U/L): 175 ± 434
  - SD: 0.70 ± 1.56
  - CV (%): 0.41 ± 0.35

**Sensitivity**: 1 U/L = 0.0003 ΔA/min

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

The results obtained using 50 samples were the following:

- Correlation coefficient (r): 0.99938
- Regression equation: y=1.025x - 1.105
- Measuring range:
  - 0,61 U/L
  - 1,0 U/L
  - 1,22 U/L

**INTERFERENCES**

Fluoride, oxalate, citrate and EDTA inhibit alkaline phosphatase activity and should therefore not be used as anticoagulants. Haemolyses interferes due to the high concentration of alkaline phosphatase in red cells.

A list of drugs and other interfering substances with acid phosphatase determination has been reported.

**BIBLIOGRAPHY**