# Alkaline phosphatase
# p-Nitrophenylphosphate. Kinetic. DGKC
# Quantitative determination of alkaline phosphatase (ALP)

IVD For *in vitro* diagnostic and professional use only

Store at 2-8°C

## INTENDE USE

For the quantitative determination of alkaline phosphatase in human serum or heparinzed plasma.

## PRINCIPLE

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'.

## 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 clinically important. 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.

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

## REAGENTS

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

## PREPARATION

Working reagent (WR)

- Dissolve one tablet of R 2 Substrate in 3ml of R 1 Buffer.
- Cap and mix gently to dissolve contents
- Stability: 21 days at 2-8°C or 5 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 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 405 nm >1.30.

## ADDITIONAL EQUIPMENT

- Spectrophotometer or colorimeter measuring at 405 nm.
- Thermostatic bath at 25°C/ 30°C / 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 | 405nm |
| Cuvette | 1 cm light path |
| Constant temperature | 25 ,30 ,37°C |

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, incubate for 1 minute.
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 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 liter of sample (U/L).

**Temperature conversion factors**

To correct results to other temperatures multiply by:

| Assay temperature | Conversion factor to | | |
|---|---|---|---|
| | 25°C | 30°C | 37°C |
| 25°C | 1.00 | 1.22 | 1.64 |
| 30°C | 0.82 | 1.00 | 1.33 |
| 37°C | 0.61 | 0.75 | 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 | 25°C | 30°C | 37°C |
|---|---|---|---|
| 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 1/10 with NaCl 9 g/L and multiply the result by 10.

**Precision:**

| | Intra-assay (n=20) | |
|---|---|---|
| Mean (U/L) | 174 | 443 |
| SD | 0.72 | 1.56 |
| CV (%) | 0.41 | 0.35 |

| Inter-assay (n=20) | |
|---|---|
| 175 | 434 |
| 6.88 | 11.93 |
| 3.93 | 2.75 |

**Sensitivity:**

1 U/L = 0.0003 ΔA /min.

**Accuracy:**

Results obtained using ATLAS 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 $(r)^2$: 0.99938.

Regression equation: y= 1.025x – 1.105.

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

**INTERFERENCES**

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.

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

**REFERENCES**


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| | | | |
|---|---|---|---|
| REF | Catalogue Number | | Temperature limit |
| IVD | *In Vitro* diagnostic medical device | | Caution |
| Σ | Contains sufficient for <n> tests and Relative size | | Consult instructions for use (IFU) |
| LOT | Batch code | | Manufacturer |
| | Fragile, handle with care | | Use-by date |
| | Manufacturer fax number | | Do not use if package is damaged |
| | Manufacturer telephone number | | Date of Manufacture |
| | Keep away from sunlight | | Keep dry |