Alkaline phosphatase (ALP)
Kinetic test optimised (DGKC)

Code HBE02 15 x 15 ml

Store at 2-8°C.

Clinical significance
Distributed in almost every tissue of the body, serum alkaline phosphatase (ALP) levels are of interest in the diagnosis of hepatobiliary disorder and bone disease. Most of the ALP in the normal adult serum is from the liver or biliary tract. Normal alkaline phosphatase levels are age-dependent, and are elevated during periods of active bone growth.

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:

\[ \text{ALP} \quad \text{p-nitrophenyl phosphate} + \text{H}_2\text{O} \rightarrow \text{p-nitrophenol} + \text{phosphate} \]

Reagent composition
- Reagent 1: Diethanolamine buffer pH 10.4 1 mmol/l
- Reagent 2: Magnesium chloride 0.5 mmol/l
- Substrate: p-nitrophenol phosphate 10 mmol/l

For in vitro diagnostic use only.

Preparation
Dissolve one tablet R.2. in 15 ml of buffer Reagent R.1. Cap and mix gently to dissolve the contents.

The stability of working reagent is 5 days at room temperature (15–25°C) or 21 days at 2-8°C.

Storage and Stability
All the components of the kit are stable at 2-8°C up to the date of expiration as specified, when stored tightly closed, protected from light and contaminations prevented during their use. Do not use the tablets if they appear to be broken.

The reagent should be a clear solution. If turbidity or precipitation has occurred or if blank absorbance at 405 nm is ≥1.30, the reagent should be discarded.

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 heparinized plasma. Use unhemolyzed serum, separated from the clot as soon as possible.

Stability: 3 days at 2-8°C

Procedure
1. Wavelength 405 nm; Temperature 25, 30, 37°C; Cuvette 1 cm light path.
2. Adjust the instrument to zero with distilled water or air.
3. Pipette into a cuvette:
   - Working reagent: 1.2 ml
   - Sample: 20 µl

Mix and wait 1 min. Read initial absorbance (abs), start the stopwatch and read absorbances every minute for 3 min. Calculate the difference between the absorbances and the average absorbance differences per minute (Δ-abs./min).

Calculation
ALP (U/l) = Δ-abs/min x 3300

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:

<table>
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<th>Assay Temperature</th>
<th>Desired temperature</th>
<th>25°C</th>
<th>30°C</th>
<th>37°C</th>
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<td></td>
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<td></td>
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</table>

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 calibrator for problems. Each laboratory should establish its own QC scheme and corrective actions if controls do not meet the acceptable tolerances.

Normal and pathological human (HBC01, HBC02) or bovine (HBC04, HBC05) sera are available.

Reference values

| Factors affecting ALP activities in a normal population include exercise, periods of growth in children and pregnancy. These values are for orientation purpose. Each laboratory should establish its own reference range. |
|---|---|---|
| Children (1-14 years) | 25°C | 30°C | 37°C |
| <400 U/l | <480 U/l | <645 U/l |
| Adults | 60-170 U/l | 73-207 U/l | 98-279 U/l |

Performance characteristics

| Measuring range: from 4,26 U/l (detection limit) to 825 U/l (linearity limit). If the obtained results are greater than 825 U/l, dilute the sample 1:2 with saline solution, repeat the determination, and multiply the result by factor 2. |
|---|---|---|
| Precision: | Intra-assay (n=20) | Inter-assay (n=20) |
| Mean (U/l) | 175 | 176 |
| SD | 2.28 | 4.60 |
| CV (%) | 1.30 | 2.61 |
| Sensitivity: | 1 U/l = 0.0003 ΔAbs/min |
| Accuracy: | Results obtained using CYPRESS DIAGNOSTICS reagents did not show systematic differences when compared with other commercial reagents. |

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

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

Bibliography
Young DS, Effects of diseases on Clinical Lab. Tests, 4th ed AACC 2001