Alkaline phosphatase FS*  
DGKC

Diagnostic reagent for quantitative in vitro determination of alkaline phosphatase (ALP) in serum or plasma on photometric systems

Order Information

<table>
<thead>
<tr>
<th>Cat. No.</th>
<th>Kit size</th>
<th>1</th>
<th>0401 99 10 021</th>
<th>R1</th>
<th>5 x 20 mL + R2 1 x 25 mL</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>1</td>
<td>0401 99 10 026</td>
<td>R1</td>
<td>5 x 80 mL + R2 1 x 100 mL</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1</td>
<td>0401 99 10 023</td>
<td>R1</td>
<td>1 x 800 mL + R2 1 x 200 mL</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1</td>
<td>0401 99 10 704</td>
<td>R1</td>
<td>8 x 50 mL + R2 8 x 12.5 mL</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1</td>
<td>0401 99 10 930</td>
<td>R1</td>
<td>4 x 20 mL + R2 2 x 10 mL</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1</td>
<td>0401 99 90 314</td>
<td>R1</td>
<td>10 x 20 mL + R2 2 x 30 mL</td>
</tr>
</tbody>
</table>

Summary [1,2]

Alkaline phosphatase (ALP), a hydrolytic enzyme acting optimally at alkaline pH, exists in blood in numerous distinct forms which originate mainly from bone and liver, but also from other tissues as kidney, placenta, testes, thymus, lung and tumors. Physiological increases are found during bone growth in childhood and in pregnancy, while pathological increases are largely associated with hepatobiliary and bone diseases. In hepatobiliary disease they indicate obstruction of the bile ducts as in cholestasis caused by gall stones, tumors or inflammation. Elevated activities are also observed in infectious hepatitis. In bone diseases elevated ALP activities originate from increased osteoblastic activity as in Paget’s disease, osteomalacia (rickets), bone metastases and hyperparathyroidism.

Method

Kinetic photometric test, optimized standard method according to the German Society of Clinical Chemistry (DGKC).

Principle

p-Nitrophenylphosphate + H₂O → ALP → Phosphate + p-Nitrophenol

Reagents

Components and Concentrations

R₁: Diethanolamine pH 9.8 1.2 mol/L
Magnesium chloride 0.6 mmol/L
R₂: p-Nitrophenylphosphate 50 mmol/L

Storage Instructions and Reagent Stability

The reagents are stable up to the end of the indicated month of expiry, if stored at 2 – 8°C and contamination is avoided. Do not freeze the reagents! Protect reagents from light!

Waste Management

Please refer to local legal requirements.

Materials required but not provided

NaCl solution 9 g/L; General laboratory equipment

Warnings and Precautions


2. Reagent 2 contains sodium azide (0.95 g/L) as preservative. Do not swallow! Avoid contact with skin and mucous membranes.

3. During reaction p-nitrophenol is produced which is poisonous when inhaled, swallowed or absorbed through skin. If the reaction mixture comes in contact with skin or mucous membranes wash copiously with water!

4. In very rare cases, samples of patients with gammopathy might give falsified results [7].

5. Please refer to the safety data sheets and take the necessary precautions for the use of laboratory reagents. For diagnostic purposes, the results should always be assessed with the patient’s medical history, clinical examinations and other findings.

6. For professional use only!

Reagent Preparation

Substrate Start

The reagents are ready to use.

Sample Start

Mix 4 parts of R₁ + 1 part of R₂ (e.g. 20 mL R₁ + 5 mL R₂) = mono reagent

Stability: 4 weeks at 2 – 8°C
5 days at 15 – 25°C

The mono reagent must be protected from light!

Specimen

Serum or heparin plasma

Stability [4]:
7 days at 20 – 25°C
7 days at 4 – 8°C
2 months at –20°C

Freeze only once!
Discard contaminated specimens!

Assay Procedure

Application sheets for automated systems are available on request.

Wavelength Hg 405 nm, (400 – 420 nm)
Optical path 1 cm
Temperature 25°C/30°C/37°C
Measurement Against air

Substrate Start

<table>
<thead>
<tr>
<th>Sample or calibrator</th>
<th>20 µL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reagent 1</td>
<td>1000 µL</td>
</tr>
<tr>
<td>Mix, incubate for approx. 1 min., then add:</td>
<td></td>
</tr>
<tr>
<td>Reagent 2</td>
<td>250 µL</td>
</tr>
<tr>
<td>Mix, read absorbance after 1 min. and start stopwatch. Read absorbance again after 1, 2 and 3 min.</td>
<td></td>
</tr>
</tbody>
</table>

Sample Start

<table>
<thead>
<tr>
<th>Sample or calibrator</th>
<th>20 µL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mono reagent</td>
<td>1000 µL</td>
</tr>
<tr>
<td>Mix, read absorbance after 1 min. and start stopwatch. Read absorbance again after 1, 2 and 3 min.</td>
<td></td>
</tr>
</tbody>
</table>
Calculation

With factor

From absorbance readings calculate $\Delta A/\text{min}$ and multiply by the corresponding factor from table below:

$$\Delta A/\text{min} \times \text{factor} = \text{ALP activity [U/L]}$$

With calibrator

$$\text{ALP [U/L]} = \frac{\Delta A/\text{min Sample}}{\Delta A/\text{min Calibrator}} \times \text{Conc. Calibrator [U/L]}$$

Conversion factor

$$\text{ALP [U/L]} \times 0.0167 = \text{ALP [µkat/L]}$$

Calibrators and Controls

For the calibration of automated photometric systems, DiaSys TruCal U calibrator is recommended. This method is traceable to the molar extinction coefficient. For internal quality control, DiaSys TruLab N and P control should be assayed. Each laboratory should establish corrective action in case of deviations in control recovery.

<table>
<thead>
<tr>
<th>Cat. No.</th>
<th>Kit size</th>
</tr>
</thead>
<tbody>
<tr>
<td>TruCal U</td>
<td>5 9100 99 10 063</td>
</tr>
<tr>
<td>TruLab N</td>
<td>5 9000 99 10 062</td>
</tr>
<tr>
<td>TruLab P</td>
<td>5 9050 99 10 062</td>
</tr>
</tbody>
</table>

Performance Characteristics

Measuring range

On automated systems the test is suitable for the determination of alkaline phosphatase activities up to 4500 U/L.

In case of a manual procedure, the test is suitable for alkaline phosphatase activities which correspond to a maximum of $\Delta A/\text{min}$ of 0.25.

If such value is exceeded, the sample should be diluted 1 + 9 with NaCl solution (9 g/L) and the results multiplied by 10.

Specificity/Interferences

No interference was observed by ascorbic acid up to 30 mg/dL, bilirubin up to 40 mg/dL, hemoglobin up to 150 mg/dL and lipemia up to 2000 mg/dL triglycerides. For further information on interfering substances refer to Young DS [5].

Sensitivity/Limit of Detection

The lower limit of detection is 3 U/L.

Reference Ranges [6]

<table>
<thead>
<tr>
<th>Children</th>
<th>25°C</th>
<th>30°C</th>
<th>37°C</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 – 12 year(s)</td>
<td>[U/L]</td>
<td>&lt; 480</td>
<td>&lt; 506</td>
</tr>
<tr>
<td>[µkat/L]</td>
<td>&lt; 8.00</td>
<td>&lt; 9.93</td>
<td>&lt; 12.1</td>
</tr>
<tr>
<td>13 – 17 year female</td>
<td>[U/L]</td>
<td>&lt; 296</td>
<td>&lt; 367</td>
</tr>
<tr>
<td>[µkat/L]</td>
<td>&lt; 4.93</td>
<td>&lt; 6.12</td>
<td>&lt; 7.47</td>
</tr>
<tr>
<td>male</td>
<td>[U/L]</td>
<td>&lt; 617</td>
<td>&lt; 767</td>
</tr>
<tr>
<td>[µkat/L]</td>
<td>&lt; 10.3</td>
<td>&lt; 12.8</td>
<td>&lt; 15.6</td>
</tr>
<tr>
<td>Adults</td>
<td>[U/L]</td>
<td>&lt; 170</td>
<td>&lt; 211</td>
</tr>
<tr>
<td>[µkat/L]</td>
<td>&lt; 2.83</td>
<td>&lt; 3.52</td>
<td>&lt; 4.30</td>
</tr>
</tbody>
</table>

Each laboratory should check if the reference ranges are transferable to its own patient population and determine own reference ranges if necessary.

Literature