Alkaline phosphatase FS*
IFCC mod. 37 °C

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

Order Information

<table>
<thead>
<tr>
<th>Cat. No.</th>
<th>Kit size</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 0441 99 10 021</td>
<td>R1 5 x 20 mL + R2 1 x 25 mL</td>
</tr>
<tr>
<td>1 0441 99 10 026</td>
<td>R1 5 x 80 mL + R2 1 x 100 mL</td>
</tr>
<tr>
<td>1 0441 99 10 023</td>
<td>R1 1 x 800 mL + R2 1 x 200 mL</td>
</tr>
<tr>
<td>1 0441 99 10 704</td>
<td>R1 8 x 50 mL + R2 8 x 12.5 mL</td>
</tr>
<tr>
<td>1 0441 99 10 917</td>
<td>R1 8 x 60 mL + R2 8 x 15 mL</td>
</tr>
<tr>
<td>1 0441 99 10 930</td>
<td>R1 4 x 20 mL + R2 2 x 10 mL</td>
</tr>
<tr>
<td>1 0441 99 90 314</td>
<td>R1 10 x 20 mL + R2 2 x 30 mL</td>
</tr>
</tbody>
</table>

Summary [1,2]
Alkaline phosphatase (AP), 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 AP activities originate from increased osteoblastic activity as in Paget’s disease, osteomalacia (rickets), bone metastases and hyperparathyroidism.

Method
Kinetic photometric test, according to the International Federation of Clinical Chemistry and Laboratory Medicine (IFCC).

Principle
\[ p\text{-Nitrophenylphosphate} + H_2O \overset{\text{AP}}{\longrightarrow} \text{Phosphate} + p\text{-Nitrophenol} \]

Reagents

<table>
<thead>
<tr>
<th>Components and Concentrations</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>R1</strong>: 2-Amino-2-methyl-1-propanol pH 10.4  1.1 mol/L</td>
</tr>
<tr>
<td>Magnesium acetate  2 mmol/L</td>
</tr>
<tr>
<td>Zinc sulphate  0.5 mmol/L</td>
</tr>
<tr>
<td>HEDTA  2.5 mmol/L</td>
</tr>
<tr>
<td><strong>R2</strong>: p-Nitrophenylphosphate  80 mmol/L</td>
</tr>
</tbody>
</table>

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!

Waste Management
Please refer to local legal requirements.

Materials required but not provided
NaCl solution 9 g/L
General laboratory equipment

Warnings and Precautions
1. The reagents contain sodium azide (0.95 g/L) as preservative. Do not swallow! Avoid contact with skin and mucous membranes.
2. 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!
3. In very rare cases, samples of patients with gammopathy might give falsified results [9].
4. 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.
5. 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) = monoreagent

Stability
4 weeks at 2 – 8 °C
5 days at 15 – 25 °C
The monoreagent must be protected from light.

Specimen
Serum or heparin plasma
Do not use hemolytic samples!

Stability [4]: 7 days at 20 – 25 °C
7 days at 4 – 8 °C
2 months at –20 °C
Only freeze 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 37 °C
Measurement Against reagent blank

| Substrate start |
|-----------------|-----------------|
| **Blank**  | Sample or calibrator |
| Dist. Water | 20 µL | 20 µL |
| Reagent 1 | 1000 µL | 1000 µL |
| Reagent 2 | 250 µL | 250 µL |

Mix, incubate for approx. 1 min., then add:
Mix, read absorbance after 1 min. and start stopwatch. Read absorbance again after 1, 2 and 3 min.
### Sample start

<table>
<thead>
<tr>
<th>Blank</th>
<th>Sample or calibrator</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>20 µL</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Dist. Water</th>
<th>Monoaagent</th>
</tr>
</thead>
<tbody>
<tr>
<td>20 µL</td>
<td>1000 µL/1000 µL</td>
</tr>
</tbody>
</table>

-Mx. read absorbance after 1 min. and start stopwatch. Read absorbance again after 1, 2 and 3 min.

### Calculation

**With factor**

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

\[
\text{\( \Delta A/\text{min} \times \text{factor} = \text{AP activity [U/L]} \)}
\]

**Substrate start**

405 nm 3433

**Sample start**

405 nm 2757

**With calibrator**

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

**Calculation factor**

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

### Calibrators and Controls

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

### Performance characteristics

#### Measuring range

On automated systems the test is suitable for the determination of AP activities up to 1400 U/L.

In case of a manual procedure, the test is suitable for AP activities up to 1400 U/L.

#### Specificity/Interferences

No interference was observed by ascorbic acid up to 30 mg/dL, conjugated bilirubin up to 60 mg/dL, unconjugated bilirubin to 25 mg/dL, hemoglobin up to 100 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 2 U/L.

### Reference Range

**Adults [6]**

<table>
<thead>
<tr>
<th></th>
<th>Female [U/L]</th>
<th>Male [µkat/L]</th>
</tr>
</thead>
<tbody>
<tr>
<td>48 - 49</td>
<td>75 - 216</td>
<td>1.25 - 5.27</td>
</tr>
<tr>
<td>124 - 341</td>
<td>82 - 383</td>
<td>1.37 - 6.38</td>
</tr>
<tr>
<td>108 - 317</td>
<td>104 - 345</td>
<td>1.73 - 5.75</td>
</tr>
<tr>
<td>96 - 297</td>
<td>93 - 309</td>
<td>1.55 - 5.15</td>
</tr>
<tr>
<td>69 - 235</td>
<td>86 - 315</td>
<td>1.43 - 5.25</td>
</tr>
<tr>
<td>51 - 332</td>
<td>42 - 362</td>
<td>0.70 - 6.03</td>
</tr>
<tr>
<td>50 - 162</td>
<td>74 - 390</td>
<td>1.23 - 6.50</td>
</tr>
<tr>
<td>47 - 119</td>
<td>52 - 171</td>
<td>0.87 - 2.85</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

3. IFCC primary reference procedures for the measurement of catalytic activity concentrations of enzymes at 37 °C. Part 9: Reference procedure for the measurement of catalytic concentration of alkaline phosphatase; Clin Chem Lab Med 2001; 39(Suppl.): S 346 [abstract].

### Manufacturer

DiaSys Diagnostic Systems GmbH
Alte Strasse 9  65558 Holzheim  Germany