## ALKALINE PHOSPHATASE

### INTENDED USE

Diagnostic reagent for quantitative in vitro determination of ALP in human serum or plasma.

### CLINICAL SIGNIFICANCE

Human ALP consists of a group of enzymes which hydrolyse phosphates at an alkaline pH. ALP is found in practically all tissues of the body but in high concentrations in the osteoblasts of bone, liver, placenta, kidney, intestinal wall and lactating mammary glands. In adults the ALP normally found circulating in the serum is largely derived from the liver. In children or adolescents going through pubertal growth spurts, there is an additional contribution from bone and this accounts for the higher reference interval for these groups. Pregnancy also raises the normal values of ALP.

Raised ALP levels are often observed in bone disease or liver disease involving the biliary tract. If the source of the isoenzyme is not apparent then estimation of GGT may help differentiate between the two. A raised GGT in the presence of a raised ALP would suggest the liver is the primary source.

Increased ALP (usually normal GGT) is seen in Osteomalacia and Rickets, primary hyperparathyroidism with bone involvement, Paget’s disease, secondary carcinoma in bone and some cases of osteogenic sarcoma. Increased levels of ALP (usually with a raised GGT) are seen in cholestasis, hepatitis, cirrhosis, space occupying lesions and malignancy with bone or liver involvement or direct production. Low levels of ALP may be observed in conditions which cause arrested bone growth or in hypophosphataemia.

### PRINCIPLE

The method according to IFCC recommendation. This method utilises 4-nitrophenyl phosphate as the substrate. Under optimised conditions ALP present in the sample catalyses the following reaction involving or direct production. Low levels of ALP may be observed in conditions which cause arrested bone growth or in hypophosphataemia.

### REAGENT COMPOSITION

**R1**
- AMP buffer, pH 10.4: 434 mmol/l
- Magnesium acetate: 2.48 mmol/l
- Zinc sulfate: 1.34 mmol/l
- HEDTA: 2.48 mmol/l
- R2: p-nitrophenyl phosphate: 19.5 mmol/l

**R2**
- Mg⁺/Alkaline pH

### REAGENT PREPARATION

Reagent is liquid, ready to use.

### STABILITY AND STORAGE

The unopened reagents are stable until the expiry date stated on the bottle and kit label when stored at 2–8°C.

Two reagents method – substrate start

### ASSAY PROCEDURE

**Wavelength:** 420 (405 – 430) nm

<table>
<thead>
<tr>
<th>Reagent</th>
<th>Calibrator</th>
<th>Sample</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blank</td>
<td>0.800 ml</td>
<td>0.800 ml</td>
</tr>
<tr>
<td>Sample</td>
<td>-</td>
<td>0.020 ml</td>
</tr>
<tr>
<td>Distilled water</td>
<td>0.020 ml</td>
<td>-</td>
</tr>
</tbody>
</table>

Mix and incubate 1 min. at 37°C and then measure the initial absorbance of calibrator and sample against reagent blank. Measure the absorbance change exactly after 1, 2 and 3 min. Calculate 1 minute absorbance change (ΔA/min).

**Monoreagent method - sample start**

Mix, incubate 1 min. at 37°C and then measure the initial absorbance of calibrator and sample against reagent blank. Measure the absorbance change exactly after 1, 2 and 3 min. Calculate 1 minute absorbance change (ΔA/min).

### CALCULATION

1. \( \text{ALP (UI)} = \frac{\Delta A_{\text{cal/m}} \times C_{\text{cal}}}{f} \)
2. Using factor: \( f = \frac{\text{ΔA}_{\text{cal/m}}}{\text{ΔA}_{\text{min}}} \)

Applications for automatic analysers are available on request.

### ASSAY PARAMETERS FOR PHOTOMETERS

**Mode:** Kinetic

<table>
<thead>
<tr>
<th>Wavelength (nm)</th>
<th>Sample Volume (μl)</th>
<th>Working Reagent Volume (μl)</th>
<th>Lag time (sec.)</th>
<th>Kinetic interval (sec.)</th>
<th>No. of readings</th>
<th>Kinetic factor</th>
<th>Reaction temperature (°C)</th>
<th>Reaction direction</th>
<th>Normal Low UI</th>
<th>Normal High UI</th>
<th>Linearity Low UI</th>
<th>Linearity High UI</th>
</tr>
</thead>
<tbody>
<tr>
<td>405</td>
<td>10/20</td>
<td>500/1000</td>
<td>60</td>
<td>60</td>
<td>3</td>
<td>2764</td>
<td>37</td>
<td>Increasing</td>
<td>42</td>
<td>128</td>
<td>3.2</td>
<td>1080</td>
</tr>
</tbody>
</table>

### COMPARISON

A comparison between XL-Systems Amylase (y) and a commercially available test (x) using 40 samples gave following results:

- \( y = 0.947 x - 3.60 \text{ UI} \)
- \( r = 0.996 \)

### INTERFERENCES

Following substances do not interfere: haemoglobin up to 5 g/l, bilirubin up to 40 mg/dl, triglycerides up to 2000 mg/dl.

### WARNING AND PRECAUTIONS

For in vitro diagnostic use. To be handled by qualified and professionally educated person. Reagents of the kit contain less than 0.1% sodium azide - classified as very toxic and dangerous substance for the environment.

### SPECIMEN COLLECTION AND HANDLING

Use serum, plasma (heparin, EDTA).

It is recommended to follow NCCLS procedures (or similar standardized conditions).

### STABILITY IN SERUM / PLASMA

<table>
<thead>
<tr>
<th>Temperature</th>
<th>Stability</th>
</tr>
</thead>
<tbody>
<tr>
<td>20–25°C</td>
<td>4 hours</td>
</tr>
<tr>
<td>20°C</td>
<td>3 days</td>
</tr>
<tr>
<td>4–8°C</td>
<td>2 months</td>
</tr>
</tbody>
</table>

### WASTE MANAGEMENT

Please refer to local legal requirements.
REFERENCES
1. Zilva JF, Pannall PR, “Plasma Enzymes in Diagnosis” in Clinical Chemistry in Diagnosis and Treatment. Lloyd London 1979; Chapter 15 : 343.