Alkaline phosphatase (ALP) Liquizyme (1 + 1) IFCC E.C.3.1.3.1.

**Intended Use**

Spectrum Diagnostics liquizyme Alkaline Phosphatase reagent is intended for the in-vitro quantitative, diagnostic determination of ALP in human serum on both automated and manual systems.

**Background**

Alkaline phosphatase (ALP) catalyzes the hydrolysis of a wide variety of physiologic and non-physiologic phosphoric acid esters in alkaline medium (pH optimum 10). The liver and biliary tract are the source of alkaline phosphatase in normal sera. Normal alkaline phosphatase levels are age dependent being higher in children and adolescents in comparison to adults. ALP is one of the tests of choice for evaluating cholestasis and obstructive jaundice. Elevated levels are found in many diseases including hepatitis, cirrhosis, malignancy, and in bone diseases.

**Method**

Kinetic method according to the International Federation of Clinical Chemistry (IFCC) [3].

**Assay Principle**

Alkaline phosphatase (ALP) hydrolyzes p-Nitrophenylphosphate (p-NPP) to p-Nitrophenol and phosphate.

\[ p\text{-Nitrophenylphosphate} + H_2O \xrightarrow{\text{ALP}} p\text{-Nitrophenol} + \text{Phosphate} \]

The increase of absorbance per minute at 405 nm is proportional to the enzyme activity.

**Reagents**

- **Reagent 1 (R1 Buffer)**
  2-Amino-2-Methyl-1-Propanol (pH 10.3) 2.0 mol/L
  MgCl₂ 2.0 mmol/L

- **Reagent 2 (R2 Substrate)**
  p-Nitrophenyl/phosphate 16 mmol/L

For further information, refer to the Alkaline phosphatase reagent material safety data sheet.

**Precautions and Warnings**

Do not ingest or inhale. In case of contact with eyes or skin; rinse immediately with plenty of soap and water. In case of severe injuries; seek medical advice immediately.

**Reagent Preparation Storage and Stability**

All reagents are stable until expiration date stated on label when stored refrigerated at 2 - 8 °C. Working solution can be prepared by adding equal volumes from R1 and R2. Stability: 1 month at 2 – 8 °C or 5 days at 15-25°C.

**Deterioration**

Do not use liquizyme ALP reagent if it is turbid or if the absorbance of the working reagent is more than 1.0 at 405 nm. Failure to recover control values within the assigned range may be an indication of reagent deterioration.

**Specimen Collection and Preservation**

**Serum and Plasma**

Nonhaemolyzed fresh serum is the preferred specimen. Heparin is the only acceptable anticoagulant. Complexing anticoagulants such as citrate, oxalate, and EDTA must be avoided. Alkaline phosphatase activity may slowly increase in serum samples stored at room temperature. Previously frozen or lyophilized sera may show a marked decrease in values immediately upon thawing or reconstitution. The activity then increases to the initial values, and the rate of this increase is time and temperature dependent.

**Stability:** 2 months at – 20 °C; 4 weeks at 4 – 8 °C; 7 days at 20 – 25 °C

**System Parameters**

- **Wavelength:** 405 nm (400 – 420 nm)
- **Optical path:** 1 cm
- **Assay type:** Kinetic
- **Direction:** Increase
- **Sample : Reagent Ratio**
  e.g.: Reagent volume 1 ml
  Sample volume 10 µl
- **Temperature:** 37 °C or 30 °C
- **Equilibration time:** 1 Minute
- **Read time:** 1 to 3 minutes
- **Zero adjustment:** Against air
- **Reagent Blank Limits**
  Low 0.2 AU
  High 1.0 AU
- **Sensitivity:** 5 U/L
- **Linearity:** 750 U/L

**Procedure**

Pipette in a test tube:

- **Working solution**
  1.0 ml (or add 0.5 ml R1 + 0.5 ml R2)
- **Specimen**
  10 µl

Mix, read initial absorbance after 1 minute, and start timer simultaneously. Read again after 1, 2 and 3 minutes. Determine the mean absorbance change per minute (ΔA/min).

**Calculation**

To calculate the alkaline phosphatase (ALP) activity. Use the following formulae

\[ U/L = 5454 \times \frac{\Delta A}{405 \text{ nm} / \text{min}} \]

**Quality Control**

Normal & abnormal commercial control serum of known concentrations should be analyzed with each run.

**Performance Characteristics**

**Precision**

Within run (Repeatability)

<table>
<thead>
<tr>
<th></th>
<th>Level 1</th>
<th>Level 2</th>
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<tbody>
<tr>
<td>n</td>
<td>20</td>
<td>20</td>
</tr>
<tr>
<td>Mean (U/L)</td>
<td>177.7</td>
<td>359.7</td>
</tr>
<tr>
<td>SD</td>
<td>1.71</td>
<td>1.5</td>
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<tr>
<td>CV%</td>
<td>0.96</td>
<td>0.43</td>
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Methods Comparison
A comparison between Spectrum Diagnostics ALP reagent and a commercial reagent of the same methodology was performed on 20 human sera. A correlation of 0.990 was obtained.

Sensitivity
When run as recommended, the minimum detection limit of this assay is 5.0 U/L.

Linearity
The reaction is linear up to alkaline Phosphatase concentration of 750 U/L; specimens showing higher concentration should be diluted 1+5 with physiological saline and repeat the assay (result×6).

Interfering Substances
Serum, plasma
Haemolysis
A 200 mg/dL haemoglobin results in a 10 % negative bias.

Icterus
No significant interference up to bilirubin level of 40 mg/dL.

Lipemia
No significant interference from lipemia up to 1000 mg/dL.

Expected Values

<table>
<thead>
<tr>
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<th>30°C</th>
<th>37°C</th>
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</thead>
<tbody>
<tr>
<td>Males (20 - 50) years</td>
<td>30 - 90 U/L</td>
<td>53 - 128 U/L</td>
</tr>
<tr>
<td>Males (&gt; 60) years</td>
<td>30 - 90 U/L</td>
<td>56 - 119 U/L</td>
</tr>
<tr>
<td>Females (20 - 50) years</td>
<td>20 - 80 U/L</td>
<td>42 - 98 U/L</td>
</tr>
<tr>
<td>Females (&gt; 60) years</td>
<td>40 - 111 U/L</td>
<td>53 - 141 U/L</td>
</tr>
<tr>
<td>Children (1 - 12) years</td>
<td>&lt; 350 U/L</td>
<td>&lt;460 U/L</td>
</tr>
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Temperature conversion factor is 1.22 (25 → 30°C) and 1.52 (25 → 37°C).

Analytical Range
5 – 750 U/L.

Waste Disposal
This product is made to be used in professional laboratories. Please consult local regulations for a correct waste disposal.

S56: dispose of this material and its container at hazardous or special waste collection point.

S57: use appropriate container to avoid environmental contamination.

S61: avoid release in environment. Refer to special instructions/safety data sheets.