Alkaline Phosphatase - Colorimetric

REF: 216 001 50 Test

Intended Use
Spectrum Diagnostics Alkaline Phosphatase Colorimetric 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
ALP – (Colorimetric method).

Assay Principle
Colorimetric determination of alkaline Phosphatase activity according to the following reaction:

\[
\text{Phenylphosphate} \rightarrow \text{ALP} \rightarrow \text{Phenol + Phosphate}
\]

Phenol liberated is measured in the presence of 4-aminoantipyrine and Potassium ferricyanide. The presence of sodium arsenate in the reagent stops the enzymatic reaction.

Reagents
Reagent 1 (R1 Buffer) pH 10
- Disodium phenylphosphate: 5.0 mmol/L
- Carbonate-bicarbonate buffer: 50 mmol/L

Reagent 2 (R2 Standard)
- Phenol: Equal to 20 kind and king U

Reagent 3 (R3 Blocking reagent)
- 4-aminoantipyrine: 60 mmol/L
- Sodium arsenate: 240 mmol/L

Buffer pH 10

Toxic reagent
R 45 : may cause cancer.
R 23/25 : toxic by inhalation and if swallowed.
S 28 : after contact with skin, wash immediately with plenty of water.
S 45 : in case of accident or if you feel unwell, seek medical advice immediately (show the label when possible).

Reagent 4 (R4 Color reagent)
- Potassium ferricyanide: 150 mmol/L

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
The reagents are supplied ready-to-use and stable up to the expiry date labeled on the bottles when stored at 2 – 8 °C.

Specimen Collection and Preservation
Serum and Plasma
Nonhemolyzed fresh serum is the preferred specimen. Heparin is the only acceptable anticoagulant. Complexing anticoagulants such as citrate, oxalate, and EDTA must be avoided.

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

System Parameters
- Wavelength: 510 nm (Hg 492)
- Optical path: 1 cm
- Direction: Increase
- Temperature: 37 °C and 20 – 25 °C

Serum Sample
Serum blank Standard Reagent blank

<table>
<thead>
<tr>
<th>Procedure</th>
<th>Serum Sample</th>
<th>Serum blank</th>
<th>Standard</th>
<th>Reagent blank</th>
</tr>
</thead>
<tbody>
<tr>
<td>R1</td>
<td>2 ml</td>
<td>2 ml</td>
<td>2 ml</td>
<td>2 ml</td>
</tr>
<tr>
<td>Incubate for 5 minutes at 37 °C</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>R2</td>
<td>Serum</td>
<td>50 µl</td>
<td>50 µl</td>
<td>50 µl</td>
</tr>
<tr>
<td>Incubate for exactly 15 minutes at 37 °C</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>R3</td>
<td>0.5 ml</td>
<td>0.5 ml</td>
<td>0.5 ml</td>
<td>0.5 ml</td>
</tr>
<tr>
<td>Mix well or preferably vortex.</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>R4</td>
<td>Serum</td>
<td>50 µl</td>
<td>50 µl</td>
<td>50 µl</td>
</tr>
<tr>
<td>Dist.Water</td>
<td>50 µl</td>
<td>50 µl</td>
<td>50 µl</td>
<td>50 µl</td>
</tr>
<tr>
<td>Mix, let stand for 10 minutes in the dark then measure. The color intensity is stable for 45 minutes.</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Calculation
\[
\text{OD serum sample} - \text{OD serum blank} \times n
\]

OD Standard
- \( n = 20 \) (Kind and king U/100 ml)
- \( n = 142 \) (IU/L)

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

Sensitivity
When run as recommended, the minimum detection limit of the assay is 1 U/100mL.

Linearity
For activities < 40 kind and king U/100 ml (285 IU/L) reassay using a smaller volume such as 20 or 10 µl. multiply the result by 2.5 or 5 respectively.
**Expected Values**

Children: 10 - 20 Kind and King U/100ml
71 - 142 IU/L

Adults: 3 - 13 Kind and King U/100ml
21 - 92 IU/L

**Note**

One Kind and King unit is the amount of enzyme which in the given conditions liberates 1 mg of phenol in 15 minutes at 37 °C

**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 instruction/safety data sheets.

**References**


---

**ORDERING INFORMATION**

<table>
<thead>
<tr>
<th>CATALOG NO.</th>
<th>QUANTITY</th>
</tr>
</thead>
<tbody>
<tr>
<td>216 001</td>
<td>50 Test</td>
</tr>
</tbody>
</table>