INTENDED USE
This reagent is intended for the quantitative determination of Alkaline Phosphatase in serum.

METHOD AND HISTORY
Alkaline phosphatase in serum is determined by measuring the rate of hydrolysis of various phosphate esters under specified conditions.

P-Nitrophenyl Phosphohydrolase is one such phosphate ester and was introduced as a substrate by Fujita in 1939. Bessey, Lowry, and Brock published an endpoint procedure in 1946 while Bowers and McComb reported a kinetic procedure in 1965. The kinetic procedure has undergone several modifications and been recommended for routine analysis. This liquid reagent is based on the recommended method of the AACC.

TEST PRINCIPLE
p-Nitrophenol Phosphohydrolase is hydrolyzed to p-nitrophenol and inorganic phosphate. The rate at which the p-NPP is hydrolyzed, measured at 405 nm, is directly proportional to the alkaline phosphatase activity.

CLINICAL SIGNIFICANCE
Serum alkaline phosphatase estimations are of interest in the diagnosis of two groups of conditions: hepatobiliary disease, and bone disease associated with increased osteoblastic activity.

SPECIMEN COLLECTION
Fresh, clear, unhemolized serum is the preferred specimen. EDTA, Oxalate and citrate inhibit the action of alkaline phosphatase. Therefore these anticoagulants should be avoided. Use a standard venipuncture tube to draw patient sample. The amount of sample required will depend on the analyzer used. The amount of serum required is in the range of 5-25 µl. Record the patient’s name, date and time of sample collection and preparation.

SPECIMEN STORAGE
Serum for alkaline phosphatase assay may be stored at room temperature (18-26°C) for up to 8 hours. Samples are stable for 4-5 days at 2-8°C and for several months at -10°C. However, it has been reported that increased activities are found after storage. It is recommended that testing be done as soon as possible after sample collection and preparation. If testing cannot occur immediately, store the sample properly using the guidelines above.

MATERIALS
Reagents necessary for the determination of alkaline phosphatase are included in the kit.

REAGENT
Alkaline Phosphatase working reagent contains:
magnesium acetate > 3.0 mM/L
p-Nitrophenyl phosphate > 11.0 mM/L
Alkaline Phosphatase buffer contains AMP buffer > 0.3 mM/L

WARNINGS AND PRECAUTIONS
For In Vitro Diagnostic Use. Not for Internal use in Humans or Animals. In Vitro Diagnostics reagents may be hazardous. Avoid ingestion and skin or eye contact.

REAGENT PREPARATION
The working reagent is prepared by mixing 4 parts of R1 to 1 part of R2.

REAGENT STORAGE AND STABILITY
Store reagent set at 2-8°C. Unopened reagents are stable until the expiration date. The working reagent is stable for 14 days at 2-8°C, and for 7 days at 18-26°C.

ADDITIONAL MATERIALS REQUIRED
A spectrophotometer or colorimeter capable of reading absorbance accurately at 405 nm. 1 cm cuvettes or a flow cell capable of transmitting light at 405 nm. Test tubes and pipettes. Timer with one minute increments. Constant temperature heat source which can be adjusted to 37°C. Normal and abnormal control for quality control.

TEST PROCEDURE
The following is a general procedure for use on a manual instrument. Application procedures for use on an automated analyzers are available. Contact King’s Technical Service Department for specific information.

PROCEDURE CONDITIONS
Wavelength 405 nm
Temperature 37°C
Pathlength 1 cm
Mode Kinetic
Lag Time 1 min.
Sample to reagent ratio 1:40

INSTRUMENT
Any instrument capable of reading absorbance accurately with a sensitivity of 0.001 absorbance at 405 nm may be used. The band width should be 10 nm or less, stray light 0.5% or less, and the wavelength accuracy within 2 nm.

CALIBRATION
No reagent calibration is necessary as the alkaline phosphatase activity is calculated by use of the molar absorptivity of p-nitrophenol which is taken as 18.8 at 405 nm.

PROCEDURE
Prepare the required number of alkaline phosphatase working reagent. Into separate test tubes pipette 25 µl of serum to be assayed. Add 1 ml of working reagent. Mix and incubate for 1 minute at 37°C. Record the absorbance at one minute intervals until the absorbance change is constant.

CALCULATION AND RESULTS
Alkaline Phosphatase U/L = \[
\Delta A / \min \times \text{assay volume (ml)} \times 1000
\]
\[
= \Delta A / \min \times 2187
\]
\[
18.8 \times \text{light path (cm)} \times \text{sample volume (ml)}
\]
\[
= \Delta A / \min \times \text{change in absorbance per minute}
\]
\[
\text{assay volume} = \text{total reaction volume expressed in ml}
\]
\[
1000 = \text{converts U/ml to U/L}
\]
\[
18.8 = \text{absorbance coefficient of p-nitrophenol at 405 nm}
\]
\[
\text{lightpath} = \text{length of the light path expressed in cm (usually 1)}
\]
\[
\text{sample volume} = \text{sample volume expressed in ml}
\]
\[
2187 = \text{factor derived from constants in the equation}
\]

Example:
Alkaline Phosphatase U/L =
\[
0.019 \times 1.025 \times 1000
\]
\[
= 0.019 \times 2187 = 42 \text{ U/L}
\]

EXPECTED VALUES
The range of expected values is: 35 - 123 U/L (37°C)
These values are suggested guidelines. It is recommended that each laboratory establish the normal range for the area in which it is located.

MEDICAL ALERT VALUES
Each laboratory should establish low and high values beyond which the patient would require immediate attention by a physician. If a "medical alert value" is reached, always repeat the test to confirm the result and notify a physician if the result is confirmed.

LIMITATIONS OF PROCEDURE

ALKALINE PHOSPHATASE
(LIQUID)
3 x 48, 3 x 12 ml
RE – ORDER ALP1010

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LIMITATIONS OF PROCEDURE

14752 FRANKLIN AVE., SUITE D, TUSTIN, CA 92780 • USA
714-734-8041 / 714-734-8036 [FAX]
A number of substances have been reported to cause physiological changes in serum alkaline phosphatase concentrations. As with any chemical reaction, users should be alert to the possible effect on results caused by unknown interferences from medications or endogenous substances. All patient results should be evaluated in light of the total clinical status of the patient.

**QUALITY CONTROL**

Standard practice for quality control should be applied to this system. Commercially available lyophilized controls can be used to monitor the daily acceptable variations. Normal and abnormal controls should be assayed at the beginning of each run of patient samples, whenever a new reagent or a different lot number is being used, and following any system maintenance. A satisfactory level of performance is achieved when the analyte values obtained are within the "acceptable range" established by the laboratory.

**CALIBRATION PROCEDURES**

No reagent calibration is necessary as the alkaline phosphatase activity is calculated by use of the molar absorptivity of p-nitrophenyl which is taken as 18.8 at 405nm.

**PRECISION**

The estimates of precision shown below were obtained from assays of human control serum.

<table>
<thead>
<tr>
<th></th>
<th>Within-Run</th>
<th></th>
<th>Between-Run</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean (U/L)</td>
<td>± 1.93</td>
<td>94</td>
<td>± 1.26</td>
</tr>
<tr>
<td>SD (U/L)</td>
<td></td>
<td>2.1</td>
<td></td>
</tr>
<tr>
<td>CV (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**CORRELATION**

A correlation study was done comparing this method and a similar alkaline phosphatase method. The samples range between 35 and 375 U/L.

<table>
<thead>
<tr>
<th>Number of Samples</th>
<th>Regression Equation</th>
<th>Correlation Coefficient</th>
</tr>
</thead>
<tbody>
<tr>
<td>95</td>
<td>y = 1.2 x + 4.5</td>
<td>0.981</td>
</tr>
</tbody>
</table>

**LINEARITY**

This procedure is linear through 1000 U/L beyond which the specimen should be diluted with an equal volume of deionized water. Reassay the specimen and multiply the results by 2.

**SENSITIVITY**

The average sensitivity for this method is 0.0003 ΔA/min per unit of concentration (U/L).

**REFERENCES**


NCCLS document "Protection of Laboratory Workers from Infectious Disease Transmitted by Blood Body Fluids, and Tissue, 2nd Ed. (1991)."