

### Intended Use

For the quantitative determination of alkaline phosphatase in human serum. For *in vitro* diagnostic use only.

### Clinical Significance

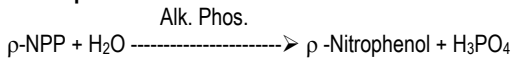
Serum alkaline phosphatase estimations are of interest in the diagnosis of two groups of conditions; hepatobiliary disease and bone disease associated with increased osteoblastic activity.<sup>1</sup>

### Test Summary

Alkaline phosphatase in serum is determined by measuring the rate of hydrolysis of various phosphate esters under specified conditions. *p*-Nitrophenyl Phosphate is one such phosphate ester and was introduced as a substrate by Fujita in 1939.<sup>2</sup>

Bessey, Lowry, and Brock published an endpoint procedure in 1946<sup>3</sup> while Bowers and McComb reported a kinetic procedure in 1966.<sup>4</sup> The kinetic procedure has undergone several modifications and been recommended for routine analysis.<sup>5,6</sup> This liquid reagent is based on the recommended method of the AACC.<sup>7</sup>

### Principle



*p*-Nitrophenyl phosphate 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.

### Reagent Composition

After combining R1 and R2 as directed the reagent contains: AMP Buffer (pH 10.45), *p*-NPP ≤16mM, Magnesium ions ≥1.0mM, activators and preservatives.

### Reagent Preparation

Mix 5 Parts of R1 Reagent with 1 part R2 Reagent.

### Reagent Storage and Stability

Store reagent set at 2-8°C. The reagents are stable until the expiration date if stored as directed. Protect from direct light and avoid microbial contamination. NOTE: The R2 reagent is temperature sensitive and can be affected by prolonged exposure to room temperature. Return reagent to 2-8°C as soon as possible after use.

### Precautions

1. This reagent set is for *in vitro* diagnostic use only.
2. Do not ingest any material, toxicity not determined.
3. Do not use if the initial absorbance of the working reagent is greater than 1.0 at 405 nm or if the reagent fails to meet the stated parameters of performance.
4. Reagent should not be used if it fails to recover stated values in control sera or shows evidence of microbial contamination.
5. All specimens and controls should be handled in accordance with good laboratory practices using appropriate precautions as described in the CDC/NIH Manual, "Biosafety in Microbiological and Biomedical Laboratories," 2<sup>nd</sup> Ed., 1988, HHS Publication No. (CDC) 88-8395.

### Specimen Collection and Storage

1. Use non-hemolyzed serum (plasma should not be used since anticoagulant agents inhibit alkaline phosphatase activity).<sup>8,9</sup>
2. Serum samples should be stored at 2-8°C and run within two days.<sup>10</sup>
3. Specimen collection should be carried out in accordance with NCCLS M29-T2.<sup>11</sup> No method can offer complete assurance that human blood samples will not transmit infection. Therefore, all blood samples should be considered potentially infectious.

### Interferences

1. Young, et al<sup>8</sup> provide a list of drugs and other substances that interfere with the determination of ALP activity.
2. The method is not influenced (< 10%) by hemoglobin values up to 500mg/dl, bilirubin levels up to 20mg/dl and lipemia / Triglycerides (Intralipid used to simulate) to 1000mg/dl. The studies were performed on the Hitachi 717™ analyzer following a modification of the guidelines contained in NCCLS document EP7-P.<sup>12</sup>

### Materials Provided

Alkaline Phosphatase R1 Reagent.

Alkaline Phosphatase R2 Reagent.

### Materials Required but not Provided

1. Accurate pipetting devices (1.0 ml and 25 ul)
2. Test tubes/rack
3. Timer (To measure one minute intervals)
4. Spectrophotometer able to read at 405 nm
5. Heating bath/block (37°C)
6. Controls to monitor the validity of the reaction

### Procedure (Automated-General)

|                       |            |
|-----------------------|------------|
| Wavelength:           | 405 nm     |
| Assay Type:           | Kinetic    |
| Sample/Reagent Ratio: | 1:41       |
| Reaction Direction:   | Increasing |
| Temperature:          | 37°C       |
| Lag Time:             | 60 seconds |
| Read Time:            | 60 seconds |
| Low Normal:           | 35 IU/L    |
| High Normal:          | 123 IU/L   |

For technical assistance concerning this product, contact the manufacturer's Technical Service Department.

### Procedure (Manual)

1. Prepare working reagent according to instructions.
2. Pipette 1.0 ml of reagent into appropriate tubes and pre-warm at 37°C for five minutes.
3. Zero spectrophotometer with water at 405 nm.
4. Transfer 0.025 ml (25 ul) of sample to reagent, mix and incubate at 37°C for one minute.
5. After one minute, read and record absorbance. Return tube to 37°C. Repeat readings every minute for the next two minutes.\*
6. Calculate the average absorbance difference per minute. ( $\Delta\text{Abs}/\text{Min}$ .)
7. The  $\Delta\text{Abs}/\text{Min}$  multiplied by the factor 2187 (See Calculation) will yield results in IU/L.

# Alkaline Phosphatase (Liquid) Reagent Set

8. Samples with values above 1000 IU/L should be diluted with an equal volume of saline, re-assayed and the results multiplied by two.

\*NOTE: If the spectrophotometer utilized is equipped with a temperature controlled cuvette, the reaction mixture may be left in the cuvette while the absorbance readings are taken.

## Limitations

1. This methodology measures total alkaline phosphatase irrespective of tissue or organ of origin. Further tests may be necessary to assist in differential diagnosis.
2. Samples with values exceeding 1000 IU/L should be diluted with an equal volume of saline and re-assayed multiplying the results by two.

## Calibration

The procedure is standardized by means of the millimolar absorptivity of p-nitrophenol (18.75 at 405nm) under the specified conditions. Results are based on the change in absorbance per unit of time; all parameters must be known and controlled.

## Calculations

One international Unit (IU/L) is defined as the amount of enzyme that catalyzes the transformation of one micromole of substrate per minute under specified conditions.

$$(IU/L) = \frac{\Delta Abs./Min. \times 1000 \times 1.025}{18.75 \times 1 \times .025} = \Delta Abs./min. \times 2187$$

Where  $\Delta Abs./Min.$  = Average absorbance change per minute  
1000 = Conversion of IU/ml to IU/L  
1.025 = Total reaction volume (ml)  
18.75 = Millimolar absorptivity of p-nitrophenol  
.025 = Sample Volume (ml)  
1 = Light path in cm

Example: If your  $\Delta Abs./min.$  = 0.06

Then  $0.06 \times 2187 = 131 IU/L$

NOTE: If test parameters are altered the factor has to be recalculated using the above formula.

SI Units: To convert to SI Units (nkat/L) multiply IU/L by 16.67.

## Quality Control

The validity of the reaction should be monitored using control sera with known normal and abnormal ALP activities and should be run with every working shift in which ALP assays are performed. It is recommended that each laboratory establish their own frequency of control determination.

## Expected Values

Adults 35-123 IU/L at 37°C. This reference range is based on a study performed by the manufacturer using samples from 783 apparently healthy adults. Children have a higher normal value. It is strongly suggested that each laboratory establish its own normal range.

## Performance (Data generated using the Technicon RA500™ analyzer)

1. Assay Range: 0-1000 IU/L
2. Correlation: A study performed between the present procedure and a similar methodology resulted in a correlation coefficient of 0.981 with a regression of  $y = 1.2x + 4.5$  (n=95, range = 35-375,  $Sy.x = 9.86$ ).
3. Precision:

| Within Run |      |       | Run to Run |      |       |
|------------|------|-------|------------|------|-------|
| Mean       | S.D. | C.V.% | Mean       | S.D. | C.V.% |
| 94         | 1.93 | 2.1   | 95         | 1.26 | 1.3   |
| 319        | 1.26 | 1.3   | 315        | 3.26 | 1.2   |

4. Sensitivity: The sensitivity for this product was investigated by reading the change in absorbance at 405nm for a saline sample and serums with known concentrations. Ten replicates were performed. The results of this investigation indicated that, on the analyzer used, the liquid alkaline phosphatase reagent showed little or no reagent drift on a zero sample. Also, that an absorbance change of 0.0003 was approximately equivalent to 1 IU/L of alkaline phosphatase activity.

## References

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12. NCCLS document "Interference testing in Clinical Chemistry", 2<sup>nd</sup> Ed. (1992).

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