POINTE SCIENTIFIC, INC.
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Alkaline Phosphatase Reagent Set

Intended Use
For the quantitative determination of Alkaline Phosphatase in human serum.

Method History
Alkaline phosphatase in serum is determined by measuring the rate of hydrolysis of various phosphate esters under specified conditions. ρ-Nitrophenyl Phosphate is one such phosphate ester and was introduced as a substrate by Fujita in 1939.[1] Bessey, Lowry, and Brock published an endpoint procedure in 1946[2] while Bowers and McComb reported a kinetic procedure in 1966.[3] In 1974, the Committee on Enzymes of the Scandinavian Society of Clinical Chemistry and Clinical Physiology adopted a modification of the above procedure as the recommended procedure.[4] The present method is based on the above two methods and that of Wilkinson, et al.[5]

Principle
$$\rho\text{-NPP} + H_2O \xrightarrow{\text{Alk Phos}} \rho\text{-Nitrophenol} + H_3PO_4$$

ρ-Nitrophenyl phosphate is hydrolyzed to ρ-nitrophenol and inorganic phosphate. The rate at which the ρ-NPP is hydrolyzed, measured at 405 nm, is directly proportional to the alkaline phosphatase activity.

Reagents
(Concentrations refer to reconstituted reagent).
Alkaline Phosphatase Reagent: ρ-Nitrophenylphosphate 10.0mM, Magnesium Ions 1.0mM, Buffer (pH 10.1±0.1), activator and binder.

Reagent Preparation
Reconstitute vial with the volume of distilled water stated on the vial label. Swirl to dissolve.

Reagent Storage
Store reagent set at 2-8°C. Reconstituted reagent is stable for sixty days when stored at 2-8°C in amber glass bottle, and seven days at room temperature.

Reagent Deterioration
Do not use reagent when:
1. The dry powder cakes because of moisture penetration.
2. The reconstituted reagent has an optical density greater than 1.0 at 405nm.

Precautions
1. This reagent is for *in vitro* diagnostic use only.
2. Avoid ingestion of all materials as toxicity has not been determined.

Specimen Collection and Storage
1. Use non-hemolyzed serum (plasma should not be used since anticoagulants inhibit alkaline phosphatase activity).[6,7]
2. Serum samples should be stored at 2-8°C and run within two days.[8]

Interferences
A number of drugs and substances affect alkaline phosphatase activity. See Young, et al.[6]

Materials Provided
Alkaline Phosphatase reagent.

Materials Required but not Provided
1. Accurate pipetting devices
2. Test tubes/rack
3. Timer
4. Spectrophotometer able to read at 405 nm (UV).
5. Heating bath/block (37°C).

Procedure (Automated)
Refer to specific instrument application instructions.

Procedure (Manual)
1. Reconstitute reagent according to instructions.
2. Pipette 1.0 ml of reagent into appropriate tubes and prewarm at 37°C for five minutes.
3. Zero spectrophotometer with water at 405nm.
4. Add 0.025ml (25ul) of sample to reagent, mix and incubate at 37°C for one minute.
5. After one minute read and record the absorbance. Return tube to 37°C. Repeat readings every minute for the next two minutes.*
6. Calculate the average absorbance difference per minute (Δabs./min.)
7. The Δabs./min. multiplied by the factor 2187 (see Calculations) will yield results in IU/L.
8. Samples with values above 800 IU/L should be diluted 1:1 with saline, re-assayed and the results multiplied by two.

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

Limitations
This methodology measures total Alkaline Phosphatase irrespective of tissue or organ of origin. Further tests may be necessary to assist in differential diagnosis.

Calibration
The procedure is standardized by means of the millimolar absorptivity of ρ-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.

Calculation
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 ΔAbs./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 ρ-Nitrophenol
. 025 = Sample Volume (ml)
1 = Light path in cm

Example: If your Δ Abs/min. = 0.06
Then 0.06 x 2187 = 131IU/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

Serum controls with known normal and abnormal values should be run routinely to monitor the validity of the reaction.

Suggested Values

Adults 35-123 IU/L at 37° C.
Children have a higher normal value. It is strongly suggested that each laboratory establish its own normal range.

Performance

1. Linearity: 800 IU/L
2. Comparison: Studies between the present method and a similar method yielded a correlation coefficient of 0.999 and a regression equation of y=0.98x-2.5.
3. Precision:

Within Run			Run to Run		
Mean	**S.D.**	**C.V.%**	**Mean**	**S.D.**	**C.V.%**
66	0.5	0.8	69	1.7	2.5
147	0.7	0.5	151	1.6	1.1

References

1. Fujita, H., J. Biochem., (Japan) 30:69 (1969).
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6. Young, D.S., et al, Clin. Chem. 15:487 (1969).
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