Alkaline Phosphatase

(King & King's Method)

Code : 10003 (15 Tests)

(For the Analyser / Colorimetric Estimation of Alkaline Phosphatase in Serum)

In VITRO USE Only.

SUMMARY & EXPLANATION OF TEST:

Phosphatase belongs to Hydrolases class of enzymes. They catalyze the splitting up of organic phosphate esters. Alakaline Phosphatase (ALP) have optimum activity at pH 10.0 these enzymes are widely distributed in the body and are present in high concentration in bone, intestine, kidney, placenta and liver. The enzyme, normally present in serum, is derived primarily from the liver and intestine with a little amount from bones. Increase in serum ALP activity is therefore an indicator of hepatobiliary or bone disorders.

ALP acts on a large number of substrates. Different investigators have used different substrates for ALP assay, e.g: Phenyl Phosphate, β-glycerophosphate, p-nitrophenyl phosphate, Phenolphthalein monophosphate, α naphthol monophosphate, etc. King and King's method in which disodium phenylphosphate is hydrolyzed by ALP with the liberation of phenol, is most commonly used. The amount of phenol liberated is proportional to ALP activity and is measured colorimetrically. Disodium phenyl phosphate has the advantage of being hydrolyzed more rapidly than β-glycerophosphate; moreover, phenol is a reactive compound which can be determined by sensitive colorimetric methods, thus permitting shorter incubation time.

Excel ALP Kit is based on the modified King and King's method, in which the buffered substrate is specially stabilized and is presented as monotest vials. In addition the color reagent is modified to reduce number of reagent additions and hence minimize errors during assay.

PRINCIPLE:

Serum ALP hydrolyzes phenyl phosphate into phenol and disodium hydrogen phosphate at pH 10.0 The phenol so formed reacts with 4-Aminoantipyrine in alkaline medium in presence of oxidizing agent Potassium ferricyanide to form a red colored complex whose absorbance is proportional to the enzyme activity.

REAGENTS:

1.	Buffered Substrate	15x 4.5 ml
2.	Color Reagent	2 x 60 ml
3.	Phenol Standard(10 KA Units)	3 ml

The reagents are ready to use and usable upto the expiration date when stored at 2-8°C.

WORKING REAGENT PREPARATION:

Reconstitute Buffered Substrate (1) with 4.5 ml of distilled water/ deionized water. Mixwell.

SAMPLE:

Serum. (ALP in Serum is stable for at least 7 days at 2-8°C.)

EXPECTED RANGE:

ALP : 3.0 - 13.0 KA Units
(Higher Values are found in children)

LINEARITY:

upto 50 KA Units

DIRECTIONS FOR USE ON ANALYSERS :

Reaction Type	:	End point with std.
Reaction Slope	:	Increasing
Wave Length	:	510 nm (green filter)
Incubation Temp	:	37°C
Incubation Time	:	3+15 min.
Standard Conc.	:	10 KA Units (King-Armstrong)
Linearity	:	50 KA Units
Unit	:	KA Units

PROCEDURE:

Pipette into clean dry tubes labeled Blank (B), Standard (S), Control (C) and Test (T) and add the reagents in the following order.

	B	S	C	T
Working Buffered Substrate	1.0ml	1.0ml	1.0ml	1.0ml
Deionized water	3.1ml	3.0ml	3.0ml	3.0ml
Incubate for 3 minutes at 37°C				
Serum	—	—	—	0.1ml
Phenol Standard (3)	—	0.1ml	—	—
Incubate for 15 minutes at 37°C				
Color Reagent (2)	2.0ml	2.0ml	2.0ml	2.0ml
Serum	—	—	0.1ml	—

Mix well after each addition of reagent and measure absorbance (A) for Blank (B), Standard (S), Control (C) and Test (T) against deionized water on photocolorimeter using a green filter or on spectrophotometer at 510 nm.

CALCULATIONS:

$$\text{Serum ALP in KA units/dl} = \frac{A(T)-A(C)}{A(S)-A(B)} \times 10 \text{ (Std. Conc.)}$$

1KA unit/dl =7.1 U/l

NOTES:

★ Due to variations in inter - laboratory assay conditions, instruments and demography, it is recommended that each laboratory should establish its own normal range. To ensure adequate quality control, each run should include a normal and abnormal assayed controls. The assigned value of the control must be confirmed by this methodology.

★ Final diagnosis should be based on a co-relation of test results with other clinical observations / Diagnostic tools.

BIBLIOGRAPHY:

1. Tietz N.W., Fundamentals of Clinical Chemistry, W.B. Saunders and company, Philadelphia, PA(1976) 602.
2. King. P.R.M & King, E.J. Clin Path 7.322.

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