

Ana-Chek® ALKALINE PHOSPHATASE

METHOD – PNPP-DEA METHOD
PRODUCT CODE – DA01



INSTRUCTIONS FOR USE

INTENDED USE: Test for estimation of Alkaline Phosphatase activity in serum / plasma using PNPP kinetic method.

SUMMARY AND PRINCIPLE

Serum Alkaline Phosphatase levels are of interest in the diagnosis of hepatobiliary disorders and bone disease associated with increased osteoblastic activity. Moderate elevations of Alkaline Phosphatase may be seen in Hodgkin's disease, congestive heart failure, ulcerative colitis, regional enteritis, and intra-abdominal bacterial infections. Ana-Chek® Alkaline Phosphatase is a reagent set for determination of Alkaline Phosphatase activity based on kinetic method using P-nitrophenyl phosphate. Ana-Chek® Alkaline Phosphatase is a single reagent system with one step reconstitution.



KIT COMPONENTS

Reagent 1: Substrate Reagent
Reagent 2: Diluent

REAGENT PREPARATION, STORAGE & STABILITY

Reconstitute each substrate tablet with diluent as per the instruction indicated on the substrate bottle. The working reagent is stable for 21 days at 2- 8 °C. The reagent kit should be stored at 2- 8 °C and is stable till the expiry date indicated on the label.

PRECAUTIONS & HANDLING

The reagents/samples should be handled by qualified personnel only. Discard reagent/sample as per good laboratory practices and local regulatory requirements. Read the instructions given on the labels and instructions for use carefully before using the kit. The kit is intended for in-vitro diagnostic use only. Don't freeze the reagent. Do not shake the reagent vigorously. Discard the reagent if the absorbance of the reagent exceeds 0.700 O.D. against D/W at 405 nm. Contamination of the reagent should be avoided.

TEST PARAMETERS

Name	ALK.PHOS.	Reagent Vol	1000 µl
Reaction Type	Kinetic (↑)	Sample Vol	20 µl
Wavelength	405 nm	Temperature	37 °C
Flow Cell Temp.	37 °C	Delay Time	60 sec.
Instrument Blank setting	D.W.	Read Time	30 sec.
Reagent Blank abs. limit	< 0.700	Factor	2720
Linearity	2000 IU/L	Standard Conc.	-

MATERIALS REQUIRED BUT NOT PROVIDED

Test tubes, Micropipette with tips, Analyzer, Controls, Incubation chamber.

SPECIMEN COLLECTION & PRESERVATION

Blood should be collected in a clean dry container. Haemolysed specimen should be avoided as it may falsely elevate results. EDTA, Citrate and Oxalate inhibit Alkaline Phosphatase activity and should not be used as anti-coagulant. Alkaline Phosphatase in the

serum/plasma is stable for 4 days when stored at 2- 8 °C and several months when stored at -10° C.

COMPONENTS OF REAGENT

Component	Concentration
Diethanolamine Buffer, pH 9.8	1 Mol/l
p-nitrophenyl phosphate	10 mmol/l
Magnesium chloride	0.5 mmol/l
Stabilizers and inactive ingredients.	-

ASSAY PROCEDURE

	Blank	Test
Reagent	1000 µl	1000 µl
Serum / Plasma	-	20 µl
Mix the reagent and sample in the above-mentioned ratio and start the stop watch.		
Aspirate reaction mixture into flow cell.		
Record absorbance at 60 th , 90 th , 120 th & 150 th sec. (30 sec. interval).		

CALCULATION

Calculate average Abs/min = Abs / 30 sec x 2
Alkaline Phosphatase (IU/L) = Abs / min x 2720

REFERENCE VALUES FOR NORMAL PEOPLE

	25 °C	30 °C	37 °C
Adult (>15 years)	60-170 IU/L	70-207 IU/L	108-306 IU/L
Children (<15 years)	150-450 IU/L	195-585 IU/L	210-810 IU/L

ACTIVITY COMPARISON CHART

Temperature of Assay	Corresponding Activity (IU/L) at		
	25 °C	30 °C	37 °C
25 °C	1.00	1.30	1.80
30 °C	0.75	1.00	1.35

PERFORMANCE CHARACTERISTICS

Measuring Range: The assay is linear between 27 - 2000 IU/L. If the Alkaline Phosphatase value exceeds linearity limit (above 2000 IU/L), dilute the specimen suitably with normal saline and repeat the assay. In that case, assay value should be multiplied with the dilution factor to obtain correct Alkaline Phosphatase value of the specimen.

Interference: There is no significant interference in samples containing Bilirubin upto 20 mg/dL and Haemoglobin upto 400 mg/dL.

Precision: Precision studies has been carried out using quality control sera as shown below:

(n=10)	Within Run			Between Run		
Specimen Material	Mean (IU/L)	SD (IU/L)	CV %	Mean (IU/L)	SD (IU/L)	CV %
Low Value Serum	105.4	0.52	0.5	110	0.92	0.8
High Value Serum	406.7	0.82	0.2	394.9	0.74	0.2

Note: We recommend all the laboratories to establish its own accuracy and precision data.

QUALITY CONTROL

Inclusion of a normal value and abnormal value chemistry control serum in each test run ensures optimum quality control. Consistent use of same type and methodology of control serum provides between run precision and accuracy data for Alkaline Phosphatase. We recommend to produce such data on daily basis for greater accuracy in assay system which include reagents, instrument, apparatus and operator.

PRECAUTIONS

1. Discard the reagent if its absorbance exceeds 0.700 at 405 nm against distilled water.
2. If Alkaline Phosphatase activity exceeds 2000 IU/L then dilute the specimen suitably with normal saline & repeat the assay. In such case the results obtained should be multiplied by dilution factor to obtain the correct Alkaline Phosphatase activity.

BIBLIOGRAPHY

1. Henry R.J., "Enzymes" in Clinical Chemistry Principle and Techniques, Harper & row Publishers, New York, 815 (1974).
2. Young D.S. et. al, Clin Chem.18,1041, (1972)

Symbol	Explanation	Symbol	Explanation
	Manufactured By		In Vitro Diagnostic Use
	Lot Number		Read Instructions Before Use
	Catalogue Number		Storage Temperature
	Manufacturing Date		Number of Tests / Volume
	Expiry Date		Do Not Reuse
	Protect from Sunlight		Keep Dry

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ISO 9001: 2015
ISO 13485: 2016
GMP
CE