

ALKALINE PHOSPHATASE

P-NPP / AMP METHOD

ORDER INFORMATION

CODE : ASH1026- R1- 2X20 ML + R2- 2X5 ML

SAFETY PRECAUTIONS AND WARNINGS :

This reagent is for *In vitro* diagnostic use only.

INTENDED USE :

This reagent kit is intended for "*in vitro*" quantitative determination of Alkaline phosphatase activity in serum.

CLINICAL SIGNIFICANCE :

Alkaline phosphatase is a membrane-bound enzyme which is present in most tissues. It has three different isoenzymes derived from small intestine - placenta - bone / liver / kidney. It is a dimer molecule containing Zn^{++} ions, which play a role in the maintenance of structure and catalysis. The enzyme found in human serum is derived from bone, liver and small intestine. During pregnancy the enzyme from the placenta dominates (it is heat stable at 65°C). In the past the iso-enzymes were separated using various inhibitors and heat. The role of electrophoresis is growing in determining the concentrations. The increase in enzyme activity is prevalent in various hepatic and bone decrease states. The level is also increased in certain diseases of the thyroid gland, intestinal tract and in several bacterial infections.

PRINCIPLE :

p-Nitrophenyl phosphate is converted to p-nitrophenol and phosphate by alkaline phosphatase. The increase of absorption at 405 nm is proportional to the alkaline phosphatase concentration in the sample.

REAGENT COMPOSITION :

Reagent 1 : AMP Buffer Reagent.
Reagent 2 : Substrate (p-Nitrophenyl Phosphate)

MATERIALS REQUIRED BUT NOT PROVIDED :

- Clean & Dry Glassware.
- Micropipettes & Tips.
- Colorimeter or Bio-Chemistry Analyzer.

SAMPLES :

Serum free of hemolysis.

WORKING REAGENT PREPARATION & STABILITY :

Mix 4 volumes of Reagent 1, with 1 Volume of reagent 2.
Working reagent is stable for 30 days at 2°- 8°C.

GENERAL SYSTEM PARAMETERS :

Reaction type	Kinetic Reaction (Increasing)
Wave length	405 nm
Light Path	1 Cm
Reaction Temperature	37°C
Blank / Zero Setting	With Distilled Water
Reagent Volume	1ml
Sample Volume	20 µl
Lag / Delay Time	60 Sec.
Read Time	90 Sec.
Interval Time	30 Sec.
Factor	2720
Linearity	1600 U/l

ASSAY PROCEDURE :

Working Reagent	1000 µl
Sample	20 µl

Mix and after 60 second incubation, measure the change in absorbance every 30 seconds for 90 seconds at 37°C.

Determine the $\Delta A/\text{min}$.

CALCULATION :

Alkaline Phosphatase Activity (U/l) = $\Delta A/\text{min} \times 2720$

LINEARITY :

Reagent is Linear up to 1600 U/l

Dilute the sample appropriately and re-assay if Alkaline Phosphatase Activity exceeds 1600 U/l or $\Delta \text{Abs} / \text{min}$ Exceeds 0.588. Multiply result with dilution factor.

REFERENCE NORMAL VALUE :

Children : 104 - 390 U/l

Adults : 25 - 140 U/l

The reference values are only indicative in nature. Every laboratory should establish its own normal ranges.

QUALITY CONTROL :

For accuracy it is necessary to run known controls with every assay.

LIMITATION & PRECAUTIONS :

1. Storage conditions as mentioned on the kit to be adhered.
2. Do not freeze or expose the reagents to higher temperature as it may affect the performance of the kit.
3. Before the assay bring all the reagents to room temperature.
4. Avoid contamination of the reagent during assay process.
5. Use clean glassware free from dust or debris.
6. Reagent to sample ratio as mentioned here above must be strictly observed as any change in it will effect the factor.

BIBLIOGRAPHY :

Fundamental of Clinical Chemistry, Young D.S, Tietz, N.
Fundamentals of Clinical Chemistry 602/609, Kaplan, M.M. New England.



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