INTRODUCTION

1. AutoZyme Alkaline Phosphatase is a reagent set for determination of Alkaline Phosphatase activity based on **kinetic method** using p-nitrophenyl phosphate (p-NPP).
2. AutoZyme Alkaline Phosphatase is a **single reagent system,** using one step procedure.
3. AutoZyme Alkaline Phosphatase has **one step reconstitution.** It involves dissolving Substrate tablet in Diluent.
4. AutoZyme Alkaline Phosphatase is a **High Stability Reagent.**
5. AutoZyme Alkaline Phosphatase is **linear** upto 700 IU/l.
6. Alkaline Phosphatase activity can be determined in just **2½ minutes.**
7. AutoZyme Alkaline Phosphatase can be used on any **Spectrophotometer, Discrete semiautomated and Automated analyzer.** Programme can be designed for any specific analyzer upon request.

PRINCIPLE

Alkaline Phosphatase cleaves p-nitrophenyl phosphate (p-NPP) into p-nitrophenol and phosphate. p-nitrophenol is a yellow colour compound in alkaline medium and absorbs light at 405 nm. The rate of increase in absorbance at 405 nm. is proportional to Alkaline phosphatase activity in specimen.

p-nitrophenyl phosphate $\xrightarrow{\text{ALK. PHOS.}}$ **p-nitrophenol + phosphate**

PREPARATION OF WORKING SOLUTION

Reconstitute each substrate tablet with diluent as per the instruction indicated on the substrate bottle.

REAGENT STORAGE & STABILITY

The reagent kit is stable till the expiry date stated on the label, when stored at 2 - 8°C.

The working solution is stable for 21 days at 2 - 8°C.

The working solution should be prepared and stored in the dark (working solution bottle) provided. This is critical because the reagent is light sensitive. It should therefore be kept away from direct light.

COMPONENTS & CONCENTRATION OF WORKING SOLUTION

Component	Concentration
• Diethanolamine buffer, pH 9.8	1 mol/l
• p-nitrophenyl phosphate	10 mmol/l
• Magnesium chloride	0.5 mmol/l

SPECIMEN COLLECTION & PRESERVATION

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

For plasma separation any of the following two anticoagulants may be used :

HEPARIN	200 IU/ml blood
SODIUM FLUORIDE	10 mg/ml blood

Serum / plasma should be separated immediately from cells. Alkaline Phosphatase is stable for 4 days at 2 - 8°C and several months when stored at -10°C.

PROCEDURE

- ☐ **Reaction type** ..... **Kinetic**
- ☐ **Reaction direction** ..... **Up**
- ☐ **Wavelength** ..... **405 nm.**
- ☐ **Flowcell temperature** ..... **37°C (± 0.2°C)**
- ☐ **Zero setting with** ..... **Working Solution**
- ☐ **Delay time** ..... **60 seconds**
- ☐ **No. of readings** **4**
- ☐ **Interval** ..... **30 seconds**
- ☐ **Blank absorbance limit** ..... **< 0.700 Abs.**
- ☐ **Sample volume** ..... **0.02 ml (20 µl)**
- ☐ **Reagent volume** ..... **1.0 ml**
- ☐ **Factor** **2720**
- ☐ **Linearity** ..... **700 IU/l**

Manual assay procedure

Prewarm the required amount of working solution at the required temperature 37°C before use. Perform the assay as given below :

1.0 ml procedure

Serum / Plasma	**0.02 ml (20 µl)**
Working Solution	**1.0 ml**

Mix thoroughly and transfer the assay mixture immediately to the thermostated cuvette and start the stop watch simultaneously. Record the first reading at 60" second and subsequently, three more readings with 30 seconds interval at 405 nm.

Calculation:

Calculate the average change in absorbance per minute.

Alkaline Phosphatase (IU/l) = Δ Abs. / min x 2720

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Conversion factors

Following factors can be used for conversion of IU/l from one temperature to another.

Temperature Conversion

Temperature of assay	Temperature factors		
	25°C	30°C	37°C
25°C	1.0	1.30	1.80
30°C	0.75	1.0	1.35
37°C	0.55	0.74	1.0

EXPECTED VALUES

	25°C	30°C	37°C
ADULT (> 15 years) IU/l	60-170	78-220	108-306
CHILD (< 15 years) IU/l	150-450	195-585	210-810

These expected values should be used as a guide only. It is recommended that each laboratory should establish its own expected values.

PROCEDURE LIMITATIONS

1. The working solution is considered unsatisfactory and should not be used if the absorbance exceeds 0.700 at 405 nm. against distilled water.

2. If the Δ Abs./min exceeds 0.250, the specimen should be diluted 10 times (0.1 ml serum + 0.9 ml saline) and reassayed. Multiply the result obtained by 10.

QUALITY CONTROL

To ensure adequate quality control, it is recommended that each batch should include a normal and abnormal commercial reference control serum. It should be realised that the use of quality control material checks both instrument and reagent functions together. Factors which might affect the performance of this test include proper instrument function, temperature control, cleanliness of glassware and accuracy of pipetting.

REFERENCES

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)

IVD	*In Vitro* Diagnostic Use		Date of Manufacturing
	Consult Instructions for use		Use by (YYYY-MM-DD)
REF	Catalogue Number		Temperature Limitation
LOT	Batch Code		Manufacturer

CE European Conformity

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