Manual Procedure

Automated procedure on request

MEDICHEM MIDDLE EAST
Clinical Chemistry Reagents
Liquid Stable Reagents

Alkaline Phosphatase

Kinetic colorimetric method based on IFCC recommendations

Cat. No. 14060	R1	1 X	50	ml
For 55 tests	R2	1 X	5	ml
Cat. No. 14061	R1	2 X	50	ml
For 110 tests	R2	1 X	10	ml

Liquid Reagents

Test principle

p-nitrophenylphosphate + H_2O $\xrightarrow{ALP}$ Phosphate + p-nitrophenol

Alkaline phosphatase hydrolyzes p-Nitrophenyl phosphate (p-NPP) to produce p-Nitrophenol and inorganic phosphate. The rate at which p-Nitrophenol is released, measured at 405 nm, is directly proportional to the alkaline phosphatase activity.

Concentrations in the test

Reagent R1 (pH = 10.8)		
2-amino-2-methyl-1-propanol (AMP)	1	mol/L
Magnesium acetate	2.0	mmol/L
Reagent R2		
p-Nitrophenylphosphate	18.5	mmol/L

Stability and preparation of working reagent

Reagent R1: *liquid.*
Reagent R2: *liquid.*
All reagents are stable up to expiry date given on the label when stored at +2 ➔ +8 °C.

Working Reagent:
Add 10 volumes of bottle R1 to 1 volume of bottle R2.
Stability : 1 months at 2 - 8 °C.

Note: Don't use if the initial absorbance of the working reagent is greater than 1.500 at 405 nm against distilled water.

Specimen collection and handling

1. Use non-hemolyzed serum or heparinized plasma. Other anticoagulantsnts should not be used since they inhibit alkaline phosphatase activity.
2. Samples are stable for 7 days at 2 - 8 °C.

Calibrator

MediCal U Cat. No. 15011

Quality control

Meditrol N Cat. No. 15171
Meditrol P Cat. No. 15181

Procedure

Wavelength	Hg 405 (400 - 420 nm)
Spectrophotometer	410 nm
Cuvette	1 cm light path
Temperature	37°C
Measurement	against air or distilled water
Reaction	kinetic – increase

Assay: Incubate working Reagent at 37°C before use:

Sample	20 µl
Working Reagent	1000 µl
Mix, incubate at 37°C for 60 sec. Read change in the absorbance per 1 min for 3 min. Determine the mean absorbance change per 1 min (ΔA/min).	

Calculation

Alkaline Phosphatase activity in sample (U/L) = (ΔA/min.) x Factor

Factors

Wavelength	405 nm	410 nm
Factor at 37°C	2764	2910

Note: It is recommended that each laboratory (as per instrument performance) could make its own factor (F) by the use of a calibrator according to the following formula:

$$F = \frac{Conc_{calibrator}}{\Delta A/\min_{Calibrator}}$$

Linearity

Up to 600 U/L at 405 nm (37°C).
If the resutl higher than 600 U/L, Repeat the test using diluted serum (1+4) with sodium chloride solution (0.9 %) and multiply the result by 5.

Interferences

1. Hemolysis interferes with the test.
2. Bilirubin up to 20 mg/dl was demonstrated to have negligible effect (≤ 5 %) on results.
3. provide a list of drugs and other substances that interfere with the determination of alkaline phosphatase activity.

Precautions

The reaction of the substrate librates p-nitrophenol, which is harmful. Don't swallow or inhale vapors. Avoid contact with skin. If solution comes into contact with skin, flush immediately with polyethylene glycol 400 (pharmaceutical grade) or, leaking this, with large quantities of water.

Reference range

Infant	1 - 30 d.	Up to 406 U/L
Children	1 - 12 mth.	Up to 383 U/L
Children	1 - 12 yr.	Up to 345 U/L
Women	13 - 17 yr.	Up to 187 U/L
Men	13 - 17 yr.	Up to 390 U/L
Women	20 - 50 yr.	< 98 U/L
Women	> 60 yr.	< 141 U/L
Men	20 - 50 yr.	< 128 U/L
Men	> 60 yr.	< 119 U/L

References

1. A. D. Rinker., Clin. Chem. 30/5, 704 - 706 (1984).
2. C. A. Burtis., Clin. Chem. 29/5, 751 - 761 (1983).
3. Tietz, N.W. Burtis CA, Duncan P, Ervin K, Petit Clerc J,Rinker AD, Shuey D, Zygowicz ER. A reference method for the measurement of alkaline phosphatase activity in human serum. Clin Chem 1983, 29 : 751 - 761.
4. Tietz, N. W. , Shuey DF.Clin. Chem. 32 : 1593 - 4 (1986).
5. Tietz, N. W. ; Rinker, A. D.Clin. Chem. 30 : 704 (1984).
6. Titez, N. W., and D. F. shuey. (1986). Clin Chem. 32 : 1593.
7. Young, DS., Effects of Drugs on Clinical Laboratory Tests, fifth edition 2000, AACC Press, Washington.

In vitro diagnostics

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