Alkaline phosphatase Assay Kit （ALP）

Method: IFCC

Cat .No.	Size	Instrument
GB040G	R1: 4×100 ml R2: 2×50 ml	For Hitachi 717 & ShimadzuCL7200/8000
GS041G	R1: 6×60 ml R2: 2×45 ml	For Hitachi 917 & OlympusAU640/400/600
GH041G	R1: 2×50 ml R2: 1×25 ml	For Hitachi 902
GT041G	R1: 5×48 ml R2: 2×30 ml	For TOSHIBA 40
GX041G	R1: 2×80 ml R2: 2×20 ml	For SYNCHRON CX4-5-7-9/LX20/DXC600-800

INTENDED USE

For the *in vitro* quantitative determination of alkaline phosphatase (ALP) in serum .

CLINICAL SIGNIFICANCE[1,2]

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^{2+} 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 isoenzymes 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 infection.

ASSAY PRINCIPLE[3]

Under alkaline condition, colorless p-nitrophenol is converted to 4-nitrophenoxide, which develops a very intense yellow color. Its intensity is proportional to the activity of alkaline phosphatase.

$$\text{p-nitrophenyl phosphate} + H_2O \xrightarrow{\text{Alkaline phosphatase}} \text{p-nitrophenol} + \text{phosphate}$$

SAMPLE COLLECTION AND PREPARATION

Serum: Use only non haemolysed serum.
Other anticoagulants interfere with the test.
Alkaline Phosphatase increases slowly with storage. Samples are stable for 4 days when stored at 2-8℃. ALP concentration is increased after meals, hence sample should be collected under fasting condition.

REAGENT COMPOSITION

Contents	Concentration of Solutions
Reagent 1 (R1)	
Magnesium sulphate	2.0 mmol/L
Zinc sulphate	1.0 mmol/L
EDTA	2.0 mmol/L
2-Amino-2-Methyl-1-Propanol (pH=10.4)	0.35 mol/L
Reagent 2 (R2)	
p-Nitrophenlphosphate	16 mmol/L

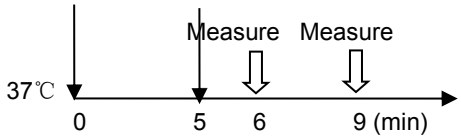
STABILITY AND PREPARATION OF REAGENTS

All reagents are ready to use.
Stable up to the expiry date when stored at 2-8℃.

ASSAY PROCEDURE

Test Procedure for Analyzers (HITACHI 917)
Assay Mode: 2 Point Rate 22-29
Wave Length (main/sub): 405 nm/600 nm

Sample: 4 µl
R1: 200 µl R2: 50 µl



1. Mix 4 µl sample with 200 µl R1 and incubate at 37℃ for 5 minutes.
2. Add 50 µl R2 into cuvette, mix and incubate at 37℃ for 1 minute.
3. Read initial absorbance and start timer simultaneously, read again after 1, 2 and 3 minutes.
4. Calculate absorbance change per minute (ΔA/min)

CALCULATION

1.Calculation using calibration

$$\text{Concentration} = \frac{A_{sample}\ /\text{min}}{A_{calibrator}/\text{min}} \times \text{Calibrator value}$$

2. Calculation using factor (K)

$$\text{ALP (U/L)} = \frac{\Delta A/\text{min} \times V_t}{\varepsilon \times V_s \times L} \times 1000 = \Delta A/\text{min} \times K$$

K = 3433 ε=18.5

CALIBRATION

Recommend that this assay should be calibrated using Randox Calibration Serum Level 3 or Level 2.

QUALITY CONTROL

Randox Assayed Multisera, Level 2 and Level 3 are recommended for daily quality control. Two levels of controls should be assayed at least once a day. Values obtained should fall within a specified range. If these values fall outside the range and repetition excludes error, the following steps should be taken:

1. Check instrument settings and light source.
2. Check reaction temperature.
3. Check expiration date of kit and contents.

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NORMAL VALUE[5,6]

MAN Women

1~12years oid

45-135 (U/L)

It is recommended that each laboratory establish its own reference range to reflect the age, sex, diet and geographical location of the population.

SPECIFIC PERFORMANCE CHARACTERISTICS

LINEARITY

When run as recommended the assay is linear up to 1702 U/L. If the sample above this concentration should be diluted 1+1 with 0.9% NaCL and repeat assay. Multiply the result by 2.

PRECISION

The CV of the test should be CV ≤10%

Intra assay precision		
N=20	Level1	Level 2
Mean (U/L)	202.3	396.6
SD	0.91	2.88
CV	0.45%	0.73%
Inter assay precision		
N=5	Level1	Level 2
Mean (U/L)	203.6	396.4
SD	2.33	8.34
CV	1.15%	2.10%

SENSITIVITY

The minimum detectable level that can be distinguished from zero has been determined as 4.25 U/L.

INTERFERENCE[4]

A Reagent blank may be performed by replacing sample or standard with double deionized water. The following analyze were tested up to the levels indicated and found not to interfere:

Hemoglobin: 1000 mg/dl
Intralipid: 2000 mg/dl
Bilirubin: 50 mg/dl
Ascorbic Acid: 50 mg/dl
Glucose: 1000 mg/dl

CORRELATION

This method (Y) was compared with another commercially available method (X) and the following linear regression equation obtained:

Y=0.956X-0.314, R^2=0.998; 50 patient samples were analyzed.

SAFETY PRECAUTIONS AND WARNINGS

1. For in vitro diagnostic use only. Do not pipette by mouth. Exercise the normal precautions required for handing laboratory reagents.
2. The reagents contains Sodium Azide. Avoid ingestion or contact with skin or mucous membranes. In case of skin contact, flush affected area with copious amounts of water. In case of contact with eyes or if ingested, seek immediate medical attention.
3. Sodium Azide reacts with lead and copper plumbing, to form potentially explosive azides. When disposing of such reagents flush with large volumes of water to prevent azide build up. Exposed metal surfaces should be cleaned with 10% sodium hydroxide.
4. All specimens used in this test should be considered potentially infectious. Universal Precautions, as they apply at your facility, should be used for handling and disposing of materials during and after testing.

REFERENCES

1. Haussament T.U. et. al. Clin. Chem. Acta 35, 271-273 (1977)
2. Zilva JF, Pannall PR, “Plasma Enzymes in Diagnosis” in Clinical Chemistry in Diagnosis and Treatment. Lloyd London 1979:Chap 15 343.
3. IFCC method for the measurement of ALP J Clin Chem Clin Biochem 1983: 21:731-48.
4. Young DS. Effects of Drugs on Clinical Laboratory Tests. Third Edition 1990: 3: 19-25.
5. Tietz Textbook of Clinical Chemistry, Second Edition, WB Saunders 1994;830 - 843.
6. Wachtel M et al, Creation and Verification of Reference Intervals. Laboratory Medicine 1995; 26:593-7.

INDEX OF SYMBOLS


Manufacture


Catalogue Number


Lot number


Date of manufacture


Use by(Expiration date)


For In-Vitro Diagnostic use only


Stored at 2-8℃


Attention:See instruction for use


Authorized Representative in the European Company

6. Wachtel M et al, Creation and Verification of Reference Intervals. Laboratory Medicine 1995; 26:593-7.

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