Alkaline Phosphatase – IFCC Method

Instructions for use (IFU)

IVD CE

ORDERING INFORMATION

	Code	Composition
OPEN KONELAB INDIKO	REF B75182551	n° 10 vials x 16 mL (R.A) n° 5 vials x 8 mL (R.B)
	REF B75182552	n° 15 vials x 4 mL (R.A) n° 1 vials x 16 mL (R.B)
CHEMILAB	REF B81180211	n° 2 vials x 32 mL (R.A) n° 4 vials x 8 mL (R.B)
	REF B81180212	n° 4 vials x 31 mL (R.A) n° 4 vials x 8 mL (R.B)

INTENDED USE

Product for use in the quantitative determination in vitro of the concentration of of Alkaline Phosphatase in human serum or plasma. The results of the test must always be interpreted in conjunction with the clinical context. FOR PROFESSIONAL USE ONLY.

CLINICAL SIGNIFICANCE

Alkaline Phosphatase (ALP) is an enzyme that reacts optimally to alkaline pH. It is present in blood in numerous forms which come mainly from bone and liver, but also from other tissues such as kidneys, placenta, testicles, and lung tissue, as well as from the presence of tumors. Physiological increases occur during phases of bone growth in children and in pregnancy, which pathological increases are largely associated with hepatobiliary and bone disorders. In terms of hepatobiliary disorders, elevated activity is also observable in infectious hepatitis. The bone disorders that cause an increase in ALP activity are osteoblastic, such as Paget's disease, osteomalacia (rickets), bone metastases and hyper-para-thyroidism.

PRINCIPLE OF THE METHOD

Kinetic photometric method optimized with the International Federation of Clinical Chemistry and Laboratory Medicine (IFCC) method.

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

Storage and stability

= **Storage temperature 2-8 °C**

If stored at 2 - 8°C avoiding direct light, the reactants remain stable until the expiration date printed on the label.

Concentration

Reagent A		
	Conc.	**U.M.**
2-Amino-2-methyl-1-propanol 10.4 pH	1.1	mol/L
Magnesium Acetate	2	mmol/L
Zinc Sulfate	0.5	mmol/L
HEDTA	2.5	mmol/L
Reagent B		
p- nitrophenylphosphate	80	mmol/L

Reagents included in the kit

The reagent is described above.

Materials required but not supplied in the kit

Controls, calibrators and pipettes with adequate volume

PRECAUTIONS and WARNINGS

1. Reagents and waste materials shall be disposed of in accordance with Community waste provisions or national or regional provisions.
2. Reagents may contain non-active components such as preservatives and detergents. The total concentration of these components is below the limits set out in Regulation 1272/2008 EC and subsequent amendments and additions.
3. It is recommended that the reagent be handled in accordance with the rules of good laboratory practice and that appropriate personal protective equipment be used.
4. Do not use the reagent if it is visibly degraded (e.g. presence of corpuscles).
5. All human samples shall be handled and disposed of as potentially infectious material.
6. The kit should only be used by qualified and properly trained technical personnel.
7. Diagnoses shall be carried out exclusively by authorised and qualified personnel.
8. Comply with national directives on occupational safety and quality assurance.
9. Use equipment that complies with current regulations.

Reporting of serious incidents

In the event of a serious incident in relation to the use of the device, please inform the manufacturer (via your distributor) and the competent authority of the European Union member state where the incident occurred. For other jurisdictions, reporting must be made in accordance with regulatory requirements.
Reporting serious incidents helps provide more information about the safety of the diagnostic medical device.

PROCEDURE

Quality control

Control sera with a known titer of **ALP** are commercially available for quality control, with values and confidence limits included. . Normal and pathological control sera Sclavo Diagnostics are available: Clinicontrol N 5x5mL cod. B35181700 and Clinicontrol A 5x5 mL code B35181701. The values obtained must be within the acceptability range.

Calibration

For calibration, utilize the kit "Calibration serum" Sclavo Code B35181702.

Traceability

The ALP traceability is reported in the package insert supplied with the "Calibrator Serum".

SAMPLE

Sample types and storage

Utilize serum or plasma samples. Stability in samples for at least 7 days at room temperature, 7 days at 2-8°C and two months at -20. Utilize samples with no hemolysis.

PREPARATION OF REAGENT

Slight variations in color from batch to batch, will not affect test results.

Procedure (Monoreactive)
Add 1 volume of RB + 4 volumes RA, shake gently.
The reagent thus prepared is ready to use.
Stability: 4 weeks at 2 – 8 °C - 5 days at 15 – 25 °C
Procedure (Bireactive)
Liquid reagents are ready for use. After opening the reagents are stable until the expiry date if kept as indicated in "Storage and Stability".

Automation

The kit can be used with all automatic analysers that can meet the operating conditions of the reagent while maintaining the volumetric ratios R1/R2/C. Validated applications are available for Sclavo Konelab® - Indiko® and CHEMILAB instruments. Applications not approved by Sclavo Diagnostics do not guarantee the performance of the reagent and must therefore be approved under the responsibility of the user.

MANUAL METHOD

The kit, in Open format, can be used manually through the use of spectrophotometer or photometer with the following parameters:

Reaction conditions

Wavelength (primary):	405 nm
Temperature:	37°C
Reaction	End-point (Increasing reaction)

Technique – Monoreactive

Bring the reagents to reagent temperature and operate away from direct light.

	U.M.	Blank	Calib. Serum	Sample
Reagent	µL	1000	1000	1000
Calib. Serum	µL	-	20	-
Sample	µL	-	-	20
Water	µL	20	-	-

Mix well and after 1 minute carry out reading at 37°C.
Read the absorbance of sample (O.D. sample) and calibrator serum (O.D. calibr. serum) against reagent blank (complete reading within 3 minutes).

Technique – Procedure Bireactive

Bring the reagents to reagent temperature and operate away from direct light.

	U.M.	Calib. serum	Sample	Blank
Reagent A	µL	1000	1000	1000
Calib. serum	µL	20	-	-
Sample	µL	-	20	-
Water	µL	-	-	20
Mix well, incubate at 37°C for 1 min. and add				
Reagent B	µL	250	250	250

Mix well and after 1 minute carry out reading at 37°C. Read the absorbance of sample

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(O.D. sample) and calibrator serum (O.D. calibr. serum) against reagent blank (complete reading within 3 minutes).
The reaction volumes may be varied proportionally without alteration of results.

Results:
Manual Method
Calculation of ALP concentration:

$$\frac{\Delta \text{ D.O. Sample}}{\Delta \text{ D.O. Calibration serum}} \times \text{Calibration serum conc. (U/L)} = \text{U/L ALP}$$

Automation
The results are automatically calculated by the analyzer based on the calibration line. The analyzer automatically performs calibration in accordance with the method protocol. The calibration line is calculated automatically by the individual instruments.

Calculation of results obtained against multiplication factor

Δ D.O./min x K-factor* = U/L di ALP

K-factor Reagent B Starter = 3433
K-factor Serum Starter = 2757

REFERENCE RANGE
Adults

Women 20-50 years	U/L	42 - 98	
Men 20-50 years	U/L	53 - 128	
Women > 60 years	U/L	53-141	
Men > 60 years	U/L	56 - 119	
		Female	Male
Children			
1 – 30 days	U/L	48 – 406	75 – 316
1 month – 1 year	U/L	124 – 341	82 – 383
1 – 3 years	U/L	108 – 317	104 – 345
4 – 6 years	U/L	96 – 297	93 – 309
7 – 9 years	U/L	69 – 325	86 – 315
10 – 12 years	U/L	51 – 332	42 – 362
13 – 15 years	U/L	50 – 162	74 – 390
16 – 18 years	U/L	47 – 119	52 – 171

Each laboratory must establish its own normal-range values on the basis of its population.

CHARACTERISTICS/PERFORMANCE
Linearity
Reaction is linear up to 1400 U/L.

Accuracy - Recovery
Commercial control sera were analyzed with the kit in question following the guidelines of the CLSI protocol. The data obtained are shown in the table below.

Range	Replicate	Mean (U/L)	DS	CV%	Recovery
Low	5	162,4	0,548	0,93	105,5 %
High	5	313,6	2,074	0,66	102,8 %

Interference

Interference	Limits
Asorbic acid	**30 mg/dL**
Conjiugated Bilirubin	**60 mg/dL**
Unconjiugatec Bilirubin	**25 mg/dL**
Haemoglobin	**1000 mg/dL**
Triglycerides	**2000 mg/dL**

Precision

Accuracy in the series (Within-run precision) – Repeatability					
Range	U.M.	Average	S.D.	C.V. (%)	N
Low	U/L	68.6	0.58	0.85	20
High	U/L	243	0.97	0.40	20
Total precision (Within-lab precision)					
Range	U.M.	Average	S.D.	C.V. (%)	N
Low	U/L	69.2	1.37	1.99	20
High	U/L	238	2.40	1.01	20

Limits of sensitivity
The Sensitivity limit has been measured using serial dilutions of high concentrated sera. The smallest detectable concentration is of about 2 U/L of ALP in the conditions established for this test.

Comparison between methods
The Sclavo method for ALP IFCC was compared with a similar commercially available method, analyzing 104 human samples. The correlation data between the two methods are reported in the table below.

Parameter	Estimation
Intercept	-1.51
Slope	1.01
Correlation Coeff. (R)	0.999

Symbols used in IFU and Packaging	
IVD In vitro diagnostic medical device vitro	Manufacturer
REF Catalogue Number	Instruction for use
LOT Lot Number	Temperature limitation
Expiration date	

REFERENCES

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2. **Wroblewsky F., Ladue J.S.,** (1965). Proc. Soc. Exper. Biol and Med, 91:569
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4. **EU-Dir 1999/11** Commission Directive of 8 March 1999 adapting to technical progress the principles of good laboratory practice as specified in Council Directive 87/18/EEC.
5. **Clinical Laboratory Standards Institute (CLSI). User Verification of Performance for Precision and Trueness**; Approved Guideline – Second Edition. EP15-A2.
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REVISION	DATE	CHANGE
Rev.A	01/2023	New Issue for IVDR Regulation (UE) 2017/746 compliance

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