Alkaline Phosphatase – DGCK Method
Instructions for use (IFU)

IVD CE

ORDERING INFORMATION

	Code	Composition
OPEN KONELAB INKIDO	REF B75182550	n° 10 vials x 16 mL (R.A) n° 5 vials x 8 mL (R.B)
CHEMILAB	REF B81180201	n° 2 vials x 32 mL (R.A) n° 2 vials x 8 mL (R.B)
	REF B81180202	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 the 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 German Society of Clinical Chemistry (DGKC) method.

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

Storage and stability
= **Storage temperature 2-8 °C**

If stored closed at the indicated temperature, avoiding direct light, the intact reagents are stable until the expiration date, printed on the label.

Concentration

Reagent A			
	Conc.	U.M.	
Diethanolamine pH 9.8	1,2	mol/L	
Magnesium Chloride	0,6	mmol/L	*GHS05/GHS08
Reagent B			
p- nitrophenylphosphate	50	mmol/L	

* _*Warning*: **DANGER**
Contains_Diethanolamine (CAS 111-42-2)
H315 – Causes skin irritation.
H318 – Causes serious eye damage
H373 - May cause organ damage through prolonged exposure.
P260 - Do not breathe dust/fume/gas/mist/vapours/spray.
P280 - Wear protective gloves/protective clothing/eye protection/face protection.
P305+P351+P338 - IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.
P310 - Immediately call a POISON CENTER/doctor.
P314 - Get medical advice/attention if you feel unwell.
P501 - Dispose of contents/container in accordance with local/regional/national/international regulations.

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. Sclavo Diagnostics Normal and pathological control sera 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 value is indicated in the calibration serum package insert.

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 THE REAGENT
Slight variations in composition among batches will not affect test results.
Procedure with serum as starter
Add 1 volume of RB + 4 volumes RA, shake gently. The reagent is thus ready for use.
Stability:
4 weeks at 2 – 8 °C
5 days at 15 – 25 °C
Procedure with Reagent B as starter
The reagents are ready-to-use liquids. After opening, reagents remain stable until the expiration date if kept in the conditions indicated below in "Storage and stability".

Automation
The kit can be used with all automatic analyzers 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
Wavelength (secondary):	- nm
Temperature:	37°C
Reaction:	End-point (increment)

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Technique – Procedure with serum as starter
- Bring the reagents to room temperature and operate away from direct light.
- Reaction volumes can be varied proportionally.

	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 minutes' read absorbance at 37°C.
Read the sample and calibrator serum absorbance against blank reagent and complete reading within 3 minutes.

Technique – Procedure with Reagent B as starter

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

Mix well and after 1 minutes' read absorbance at 37°C.
Read the sample and calibrator serum absorbance against blank reagent and complete reading within 3 minutes.
Reaction volumes may be varied proportionally without alteration of results.

Results:
Manual Method
Calculation of ALP concentration:

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

Automation
The results are automatically calculated by the analyzer based on the calibration curve/line. The analyzer automatically performs calibration in accordance with the method protocol. The calibration curve/line is obtained through a special validated algorithm.

Calculation of results obtained against multiplication factor

Δ D.O./min x K-factor* = U/L of ALP

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

REFERENCE RANGE

			25°C	30°C	37°C
Adults					
Adults		U/L	< 170	< 211	< 258
Children					
1 – 12 years		U/L	< 480	< 596	< 727
13 – 17 years	Male	U/L	< 617	< 767	< 935
	Female	U/L	< 296	< 367	< 448

Every laboratory must set it own normal-range values based on the population under study.

CHARACTERISTICS/PERFORMANCE
Linearity
Reaction is linear up to 800 U/L. If the value in the sample exceeds the linearity limit of the method, dilute the sample with saline and multiply the result for the dilution factor.

Accuracy - Recovery
Accuracy studies have been carried out on normal samples added with known concentration of Alkaline Phosphatase. The data indicate a recovery of 99%.

Interference

Interference	Limits
Asorbic acid	**30 mg/dL**
Bilirubin	**40 mg/dL**
Haemoglobin	**150 mg/dL**
Triglycerides	**2000 mg/dL**

Precision of the method

Accuracy in the series (Within-run precision) – Repeatability					
Range	U.M.	Average	S.D.	C.V. (%)	N
Low	U/L	114	1.71	1.50	20
High	U/L	275	2.91	1.06	20
Total precision (Within-lab precision)					
Range	U.M.	Average	S.D.	C.V. (%)	N
Low	U/L	120	1.93	1.60	20
High	U/L	279	2.36	0.85	20

Limits of sensitivity
The Sensitivity limit has been measured using serial dilutions of high concentrated sera. The smallest detectable concentration is of about 3 U/L of ALP in the conditions established for this test.

Comparison between methods
The method was compared with a similar commercially available method, analyzing 78 human samples. The correlation data between the two methods are reported in the table below.

Parameter	Estimation
Intercept	-2.21
Slope	0.98
Correlation Coeff. (R)	0.98

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	

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REVISION	DATE	CHANGE
Rev.A	01/2023	New Issue for IVDR Regulation (UE) 2017/746 compliance

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