

# Alkaline Phosphatase – DGKC Method

Instructions for use (IFU)

# 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)			

# 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-thypoidism.

# PRINCIPLE OF THE METHOD

Kinetic photometric method optimized with the International German Society of Clinical Chemistry (DGKC) method.

p - nitropheny lphosphate  $+H_2O \xrightarrow{AP} phosphate + p - nitropheno I$ 

# 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  $2.8^{\circ}$ 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® 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)

# 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.





# Alkaline Phosphatase – DGKC Method

Instructions for use (IFU)

# 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:

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

#### 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

 $\Delta$  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		1.1/1	< 170	~ 011	< 250
Children		0/L	< 170	×211	< 200
1 – 12 years		U/L	< 480	< 596	< 727
13 – 17	Male	U/L	< 617	< 767	< 935
years	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

Accurac	Accuracy in the series (Within-run precision) – Repeatability					
Range	U.M.	Average	S.D.	C.V. (%)	Ν	
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. (%)	Ν	
Low	U/L	120	1.93	1.60	20	
High	11/1	270	2.26	0.95	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	(i) Instruction for use			
Lot Number	4 Temperature limitation			
Expiration date				

#### REFERENCES

- Thomas L. (1998) Clinical Laboratory Diagnostics. 1st ed. Frankfurt: TH-Books Verlagsgesellschaft; p. 208-14.
- Moss DW, AR Henderson (1999). Enzimologia clinica. In: Burtis CA, Ashwood ER, editori. Tietz Textbook of Chimica Clinica. 3a ed. Philadelphia: Saunders W.B Società; 1999. p. 617-721.
- Tietz NW, Rinker D, LM Shaw. (1983) IFCC metodo per fosfatasi alcalina. J Clin Chem Clin Biochem; 21:731-48.
- Tietz NW, Burtis CA, Duncan P, Ervin K, Petitclerc CJ, Rinker AD, Shuey D, Zygowicz ER (1983) A reference method for measurement of alkaline phosphatase activity in human serum. Clin chem; 29:751-61
- Guder WG, Zawta B et al. (2001) La qualità della diagnostica Campioni. 1a ed. Darmstadt: GIT Verlag; p. 14-5.
- Burtis CA, ER Ashwood. Eds. (1999) Tietz manuale di clinica chimica. 3a ed. Philadelphia: W. B. Saunders Società, p. 1829.
- JS Soldin, Brugnara C., Wong CE. (2007) In: MJ Hicks, editor. Pediatrica di riferimento intervalli. 6 ° ed. Washington: AACC Press, p. 11.
- Clinical Laboratory Standards Institute (CLSI). User Verification of Performance for Precision and Trueness; Approved Guideline – Second Edition. EP15-A2.
- Clinical Laboratory Standards Institute (CLSI). Evaluation of Precision Performance of Quantitative Measurements Methods; Approved Guideline – Second Edition. EP05-A2.
- Clinical Laboratory Standards Institute (CLSI). Measurement Procedure Comparison and Bias Estimation Using Patient Samples; Approved Guideline – Third Edition. EP09-A3.
- Clinical Laboratory Standards Institute (CLSI). Evaluation of Detection Capability for Clinical Laboratory Measurement Procedures, 2nd Edition – EP17.
- Clinical Laboratory Standards Institute (CLSI). Interference Testing in Clinical Chemistry, – Third Edition. - EP07.
- Clinical Laboratory Standards Institute (CLSI). Evaluation of Linearity of Quantitative Measurement Procedures, 2nd Edition - EP06.

REVISION	DATE	CHANGE
Rev.B	05/2025	Out of production of chemilab line



