Phosphorus – Method UV End-Point
Instructions for use (IFU)

IVD CE

ORDERING INFORMATION

	Code	Composition
OPEN KONELAB INDIKO	REF B75182508	n° 6 vials x 60 mL
	REF B75182509	n° 12 vials x 20 mL

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

CLINICAL SIGNIFICANCE
Phosphorus is an important element present in the human organism in the form of inorganic or organic phosphate. It is present in the serum in the form of both monovalent and divalent phosphate anions. About 10% of serum phosphate is bound to proteins, 35% is found in combination with sodium, calcium and magnesiun, the remaining amount is free. Phosphate represents one of the main components of hydroxyapatite which is present in bone, it has an important role as a high-energy phosphate bond and is a vital component for numerous enzymatic systems. Low blood phosphate concentrations are relatively common in the hospitalized population, due to several causes such as: stimulation of insulin secretion which favours the transport of phosphates in the cells and respiratory alkalosis. A loss of phosphates through the kidneys can cause low serum phosphate levels.

PRINCIPLE OF THE METHOD
Method UV End-Point. The method measures the absorbance at 340 nm of non-reduced phospho-molibdate and allows the determination of inorganic phosphates present in the sample. In the presence of sulphuric acid, inorganic phosphorus reacts with ammonium molibdate to give an unreduced complex which can be read at 340 nm.

Inorganic Phosphorus + H_2SO_4 + ammonium molibdate $\rightarrow$ Phospho-molibdate complex

Storage and stability
= storage temperature 2-8°C
If stored closed at 2-8 ° C, avoiding direct light, the intact reagents are stable until the expiration date, printed on the label.

Concentration

Reagent:			
	Conc.	U.M.	
Sulphuric acid	0,50	N	
Ammonium molibdate	0,61	mM	*GHS05

*_*Warning:* **DANGER**
Contains Sulphuric Acid (CAS 7664-93-9)
H314 - Causes severe skin burns and eye damage.
P303+P361+P353 - IF ON SKIN (or hair): Take off immediately all contaminated clothing. Rinse skin with water [or shower].
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.
P321 - Specific treatment (see on this label).
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 Phosphorus 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 use the "Calibrator serum Sclavo" code B35181702.

Traceability
The Inorganic Phosphorus traceability is reported in the package insert supplied with the "Calibrator Serum".

SAMPLE
Type of sample and storage
Fresh non haemolyzed serum samples should be used. Inorganic phosphorus is stable in serum for 5 days at 2-8°C and for 3 weeks at – 20°C. Urine samples must be diluted with distilled water immediately after collection, in the proportion of 1: 20 (1 part of urine with 19 parts of water) and then tested immediately. For 24-hour urine collection, add 10 ml of 10 g/dL HCl to the collection bottle to avoid phosphate precipitation. Dilute urine 1 + 10 with distinct water before determination and multiply the result by 11.

PREPARATION OF THE REAGENT
Liquid reagents are ready for use. After opening the reagents are stable until the expiry date if kept as indicated in "Storage and Stability". Slight variations in colour among batches will not affect test results.

Automation
The kit can be used with all automatic analyzers that can meet the operating conditions of the reagent while maintaining the volumetric ratios R / 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):	340 nm
Temperature:	37°C
Reaction	End-point (Increasing reaction)

Technical – procedure with Serum as starter
Bring the reagents to room temperature and operate away from direct light.

	U.M.	Blank	Calibr. Serum	Sample
Reagent	µL	1000	1000	1000
Calibr. serum	µL	-	10	-
Sample	µL	-	-	10
Blank	µL	10	-	-

Mix well and let stand for 5 minutes.
Read the absorbance of sample and calibrator serum against reagent blank. The reagent's final colour is stable for 60 minutes if protect from direct light.
The reaction volumes may be varied proportionally without alteration of results.

Results:
Manual Method
Calculation of Phosphorus concentration:

$$\frac{\text{O.D. Sample}}{\text{O.D. Calibrator serum}} \times \text{Calibrator serum Concentration} = \text{Inor. Phosphorus mg/dL}$$

Sclavo Diagnostics International S.p.A.
Loc. Pian dei Mori, via Po n° 26-28 • 53018 (SI) (Italy)
Phone +39 0577 390 41 • fax +39 0577 390 444
www.sclavodiagnostics.com

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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 different instruments.

REFERENCE RANGE
Serum
- Adults: 2.3 – 4.0 mg/dL (0.74 – 1,. mmol/L)
- Children: 4.0 – 5.4 mg/dL (1.29 – 1.74 mmol/L)

Urine
- Adults: 0.4 - 1.3 g/24 h (12.9 - 42 mmol/24 h)

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

ANALYTICAL CHARACTERISTICS/PERFORMANCE
Linearity
The method is linear up to 30 mg/dL. 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.

Recovery
Sieri di controllo commerciali sono stati analizzati con il kit in oggetto seguendo le linee guida del protocollo CLSI. I dati ottenuti sono riportati nella tabella successiva
Serum – Plasma

Range	Replicates	Mean	DS	CV%	Recovery
Basso	5	1.87	0.031	1.67	98.1 %
Alto	5	3.62	0.041	0.65	92.3 %

Urine

Range	Replicates	Mean	DS	CV%	Recovery
Basso	5	28.50	0.555	1.90	101 %
Alto	5	42.80	1.398	3.30	93.4 %

Interferences

Interference	Limite
Ascorbic Acid	**30 mg/dL**
Bilirubin	**60 mg/dL**
Haemoglobin	**1000 mg/dL**
Lipids (Triglycerides)	**2000 mg/ dl**

Please note that ditaurobilirubin interferes from the concentration of 3 mg/dL onwards, when phosphate is measured on systems, which are unable to handle a second wavelength.

Precision of the method

Accuracy in the series (Within-run precision) – Repeatability					
Range	U.M.	Mean	S.D.	C.V. (%)	No.
Low	mg/dL	3.85	0.06	1.56	30
High	mg/dL	7.15	0.12	1.68	30
Total precision (Within-lab precision)					
Range	U.M.	Mean	S.D.	C.V. (%)	No.
Low	mg/dL	3.88	0.084	2.16	20
High	mg/dL	7.26	0.152	2.09	20

Limit of Sensitivity
The Sensitivity limit has been measured using serial dilutions of high concentrated sera. The smallest detectable concentration is of about 0.10 mg/dL, at the conditions established for this test.

Comparison between methods
The methods were compared with a similar commercially available method, analyzing 60 human samples. The correlation data between the two methods are reported in the table below.

Parameter	Estimation
Intercept	-0.01
Slope	0.96
Correlation Coeff. (R)	0.998

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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4. **Amador E, Urban J. (1972)** Simplified serum phosphorus analyses by continuous- flow ultraviolet spectrophotometry J. Clin Chem 1972; 18: 601-604.
5. **Clinical Laboratory Standards Institute (CLSI).** User Verification of Performance for Precision and Trueness; Approved Guideline – Second Edition. EP15-A2.
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8. **Alan H.B. Wu (2006)** Tietz, Clinical Guide to Laboratory Test. Fourth Edition. Saunders Ed.
9. **Clinical Laboratory Standards Institute (CLSI).** Evaluation of Detection Capability for Clinical Laboratory Measurement Procedures, 2nd Edition – EP17
10. **Clinical Laboratory Standards Institute (CLSI).** Interference Testing in Clinical Chemistry, – Third Edition. - EP07
11. **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

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