DIALAB Produktion und Vertrieb von chemisch-technischen Produkten und Laborinstrumenten Gesellschaft m.b.H.
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Phosphorus Inorganic, Molybdate

(en) English

REF	Content		
D00359B	1 x	1 L	reagent
D00361	5 x	100 mL	reagent
D00362	5 x	50 mL	reagent
D00363	5 x	25 mL	reagent
D00364	5 x	10 mL	reagent
D85911	10 x	50 mL	reagent
D0435917	9 x	65 mL	reagent
DA0840	5 x	50 mL	reagent
DT1040	4 x	50 mL	reagent
DK0737	5 x	50 mL	reagent
DE1840	5 x	20 mL	reagent
DB20329	10 x	50 mL	reagent

For professional in vitro diagnostic use only.

INTENDED USE

Diagnostic reagent for quantitative in vitro determination of phosphorus in human serum, plasma or urine on photometric systems.

DIAGNOSTIC SIGNIFICANCE[1,2]

Phosphorus exists in the body almost exclusively as phosphate, mainly as inorganic substance of the bones, but also in cells in phospholipids and nucleic acids as well as in adenosine triphosphate, which is involved in the energy transfer. In plasma it is present as calcium phosphate; therefore, in level of plasma phosphorus is strongly associated with that of calcium levels.
Measurement of phosphorus in serum and urine is mainly performed to detect disorders of kidneys, bones and parathyroid glands. Increased concentrations are found in renal failure, hypoparathyroidism, pseudo-hypoparathyroidism and vitamin D deficiency.
Additional information can be obtained by supplementary measurement of calcium.

TEST PRINCIPLE

The phosphate ions react with ammonium molybdate to form a phosphomolybdate complex. The colourless phosphomolybdate complex can be measured directly by ultraviolet (UV) absorption at 340 nm. An acid pH is necessary for the formation of complexes.

REAGENT COMPOSITION

COMPONENTS	CONCENTRATION	
Ammonium molybdate	0.4	mmol/L
Sulphuric acid	0.21	mol/L
Surfactant		

MATERIAL REQUIRED BUT NOT PROVIDED

- Standard or Calibrator, e.g.:

REF	Name	Content
D95362	Phosphorus Standard	1 x 3 mL
D98485	Diacal Auto	5 x 3 mL
D98485SV	Diacal Auto	1 x 3 mL

- Controls, e.g.:

REF	Name	Content	Description
D98481	Diacon N	12 x 5 mL	Control normal
D14481	Diacon N	5 x 5 mL	Control normal
D98481SV	Diacon N	1 x 5 mL	Control normal
D98482	Diacon P	12 x 5 mL	Control abnormal
D14482	Diacon P	5 x 5 mL	Control abnormal
D98482SV	Diacon P	1 x 5 mL	Control abnormal
D08581	Diacon Urine Level 1	12 x 5 mL	Urine Ctrl. normal
D08581SV	Diacon Urine Level 1	1 x 5 mL	Urine Ctrl. normal
D08582	Diacon Urine Level 2	12 x 5 mL	Urine Ctrl. abnormal
D08582SV	Diacon Urine Level 2	1 x 5 mL	Urine Ctrl. abnormal

- NaCl solution (9 g/L).
- Photometric device.
- General laboratory equipment.
- Glass or high quality polystyrene cuvettes.

REAGENT PREPARATION

The reagent is ready to use.

STORAGE AND STABILITY

Conditions:	Close immediately after use Avoid contamination Keep way from direct light sources Do not freeze the reagent
Storage:	at 2 – 8 °C
Stability:	60 days after first opening of the primary container

WARNINGS AND PRECAUTIONS

1. Reagent may contain some non-reactive and preservative components. It is suggested to handle carefully it, avoiding contact with skin and swallow.
2. Please refer to the safety data sheets and take the necessary precautions for the use of laboratory reagents.
3. For diagnostic purposes, the results should always be assessed with the patient's medical history, clinical examinations and other findings.
4. In the event of an incident related to the device, report it to the manufacturer and your competent authority as required.
5. For professional use only!

SPECIMEN COLLECTION AND STORAGE

Serum is the preferred specimen. Although heparinized plasma is acceptable, levels of inorganic phosphate are about 0.2 to 0.3 mg/dL lower than in serum. Anticoagulants such as citrate, oxalate, and EDTA interfere with formation of the phosphomolybdate complex and should not be used.

Inorganic phosphate in whole blood specimens may either decrease or increase with time, depending on the type of specimen, temperature, and duration of storage. Levels in plasma or serum are increased by prolonged storage with cells at room temperature or 37 °C; it is important to promptly separate serum or plasma from erythrocytes. Hemolyzed specimens are unacceptable because erythrocytes contain high concentrations of organic phosphate esters, which can be hydrolyzed to inorganic phosphate during storage. Inorganic phosphate increases by 4 to 5 mg/dL per day in hemolyzed specimens stored at 4°C. Glucose phosphate, creatine phosphate, and other organic phosphates may also be hydrolyzed by assay conditions, resulting in overestimation of inorganic phosphate levels.

Phosphate is considered to be stable in serum that has been separated from the clot for days at 4°C and months when frozen.

Urine samples

Urine samples should be collected in 6 mol/L HCl, 20 – 30 mL for a 24 hours specimen, to avoid precipitation of phosphate complexes.

Sample preparation (Urine): Dilute urine samples 1:20 with purified water before assay.

STANDARD

(Not included in the kit; has to be ordered separately)

Concentration:	5 mg/dL (1.61 mmol/L)
Storage:	2 – 8 °C
Stability:	up to the indicated expiration date

Close immediately after use! Avoid contamination!

TEST PROCEDURE

Method:	UV, Endpoint, Increasing reaction, Phosphomolybdate
Wavelength:	340 nm
Optical path:	1 cm
Temperature:	25 °C, 30 °C or 37 °C

Bring reagents and samples to room temperature.

Pipette into test tubes	Blank	Std./Cal.	Sample
Reagent	1000 µL	1000 µL	1000 µL
Sample	-	-	10 µL
Std./Cal.	-	10 µL	-
Dist. water	10 µL	-	-
Mix, incubate for 5 minutes at 25, 30 or 37 °C and read absorbance against reagent blank.			

Automation

Special adaptations for automated analysers can be made on request.

INTERPRETATION OF RESULTS

Calculation

With Standard or calibrator

Serum/Plasma:

$$\text{Phosphorus [mg/dL]} = \frac{\Delta A \text{ Sample}}{\Delta A \text{ Std/Cal}} \times \text{Conc. Std/Cal [mg/dL]}$$

Random Urine sample:

$$\text{Phosphorus [mg/dL]} = \frac{\Delta A \text{ Sample}}{\Delta A \text{ Std/Cal}} \times \text{Conc. Std/Cal [mg/dL]} \times 20$$

24 hours urine sample:

$$\text{Phosphorus [g/24h]} = \frac{\frac{\Delta A \text{ Sample}}{\Delta A \text{ Std/Cal}} \times \text{Std/Cal [mg/dL]} \times 20 \times \text{urine volume}}{1000}$$

(Urine volume in decilitres)

Unit Conversion
Phosphorus [mg/dL] x 0.3229 = Phosphorus [mmol/L]
Phosphorus [mmol/L] = Phosphate [mmol/L]
Phosphorus [mg/dL] x 3.06619 = Phosphate [mg/dL]

QUALITY CONTROL AND CALIBRATION

It is suggested to perform an internal quality control.
We recommend the DIALAB serum controls **Diacon N** (control serum with values in the normal range) and **Diacon P** (control serum with values in the abnormal range) as well as the DIALAB urine controls **Diacon Urine Level 1** (control urine normal) and **Level 2** (control urine abnormal).
Each laboratory should establish corrective action in case of deviations in control recovery.

Calibration
The assay requires the use of a phosphorus standard or calibrator.
We recommend the DIALAB **Phosphorus Standard** and the DIALAB multi calibration serum **Diacal Auto.**

PERFORMANCE CHARACTERISTICS

Accuracy and precision
CV ≤ 1.90 % for within-run precision and CV ≤ 2.40 % for between-run precision.

Analytical sensitivity
The lower limit of detection is 0.4 mg/dL.

Linearity and measuring range
The test has been developed to determine phosphorus concentrations within a measuring range from 0.4 – 20 mg/dL. When values exceed this range, samples should be diluted 1 + 9 with NaCl solution (9 g/L) or distilled water (for urine samples) and the results multiplied by 10.

Analytical specificity
no interference up to:

Bilirubin	25 mg/dL
Hemoglobin	100 mg/dL

Hemolysis interferes.
Both positive and negatives interferences with lipemic samples have been observed.

Clinical performance
A comparison of DIALAB Phosphorus Inorganic, Molybdate (y) with a commercially available assay (x) using 102 samples gave following results:
$y = 1.005\ x - 0.109$ mg/dL; $r^2 = 0.975$.

Tests were performed on the following instruments: COBAS FARA II.

TRACEABILITY

The assigned values of the calibrator Diacal Auto have been made traceable to a primary phosphorus standard (traceable to the reference material NIST-SRM 723).

EXPECTED VALUES

	mg/dL	mmol/L
Serum/plasma (adults):	2.5 – 4.5	0.81 – 1.45
Serum/plasma (children):	4.0 – 7.0	1.29 – 2.26

	g/24h	mmol/24h
Urine (nonrestricted diet)	0.4 – 1.3	12.9 – 42.2

Each laboratory should check if the reference ranges are transferable to its own patient population and determine own reference ranges if necessary.

LIMITATIONS

- Eventual Phosphorus Inorganic (Molybdate) carry-over to reagents Magnesium (Xylidyl blue), Uric Acid (AOX), Uric Acid (TBHBA) and Protein Total in Urine/CSF (Pyrogallol red). The actual carry-over depends on the analyser.

WASTE MANAGEMENT

Please refer to local legal requirements.

LITERATURE

1. Thomas L. Clinical Laboratory Diagnostics. 1st ed. Frankfurt: TH-Books Verlagsgesellschaft; 1998. p.231-41.
2. Endres DB, Rude RK. Mineral and bone metabolism. In: Burtis CA, Ashwood ER. editors. Tietz Textbook of Clinical Chemistry. 3rd ed. Philadelphia: W.B. Saunders Company; 1999. p.1395-1406.
3. Yee H.Y. - Clin. Chem. 14, 898 (1968).
4. Tietz Textbook of Clinical Chemistry, Second Edition, Burtis-Ashwood (1994).

USED SYMBOLS

Symbol	Description
Cont.	Content

CE IVD