Phosphate

DiaSystem Scandinavia AB

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

Order Information

Cat. No.	Kit size						
01 00037 70 04 0100	R1	4 x	20 mL	+	R2	1 x	20 mL
01 00037 70 10 0180	R1	4 x	36 mL	+	R2	1 x	9 mL
CDT-Mg	R1	3 x	30 mL	+	R2	1 x	11,3 mL

06 00112 70 04 0018 Phosphorous Standard 6x3 mL

Summary [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, the 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-hyperparathyroidism and loss of calcium phosphate of bones and cells. Decreased values occur in malabsorption, hyperparathyroidism and vitamin D deficiency. Additional information can be obtained by supplementary measurement of calcium.

Method

Photometric UV test with endpoint determination

Principle

Ammonium molybdate + Sulphuric acid + Phosphate

→ inorg. phosphorus molybdate complex

The complex absorption is maximal at 340 nm.

Reagents

Components and Concentrations

R1:	Glycine/sulphuric acid buffer	50 mmol/L
R2:	Glycine buffer	50 mmol/L
	Ammonium molybdate	1.75 mmol/L
Standard (Phosphorus):		5 mg/dL (1.61 mmol/L)

Storage Instructions and Reagent Stability

Reagents and standard are stable up to the end of the indicated month of expiry, if stored at 2 – 8°C if contamination is avoided. Do not freeze the reagents!

Warnings and Precautions

1. Reagent 1: Warning. H290 May be corrosive to metals. P234 Keep only in original container. P280 Wear protective gloves/protective clothing/eye protection. P390 Absorb spillage to prevent material damage.
2. In very rare cases, samples of patients with gammopathy might give falsified results [7].
3. Please refer to the safety data sheets and take the necessary precautions for the use of laboratory reagents. For diagnostic purposes, the results should always be assessed with the patient's medical history, clinical examinations and other findings.
4. For professional use only!

Waste Management

Please refer to local legal requirements.

Reagent Preparation

The standard is ready to use.

Substrate Start

The reagents are ready to use.

Sample start

Mix 4 parts of R1 with 1 part of R2
(e.g. 20 mL R1 + 5 mL R2) = mono reagent.
Stability of the mono reagent:
1 year at 2 – 8 °C.

Materials required but not provided

NaCl solution 9 g/L
General laboratory equipment

Specimen

Serum, heparin plasma or urine [4]

Stability in serum/plasma:

1 day	at	20 – 25 °C
4 days	at	4 – 8 °C
1 year	at	-20 °C

Discard contaminated specimens! Only freeze once!

Stability in urine:

2 days	at	20 – 25 °C	at pH < 5

Discard contaminated specimens!

For collection of 24 h urine add 10 mL of 10 g/dL HCl into the collection bottle to avoid phosphate precipitations. Dilute urine 1 + 10 with dist. water before determination and multiply the result by 11.

Assay Procedure

Application sheets for automated systems are available on request.

Wavelength	340 nm, Hg 334 nm, Hg 365 nm 660 nm bichromatic
Optical path	1 cm
Temperature	20 - 25 °C/37 °C
Measurement	Against reagent blank

Substrate start

	Blank	Sample/ Standard
Sample/Standard	-	10 µL
Dist. water	10 µL	-
Reagent 1	800 µL	800 µL
Mix, incubate 5 min., read absorbance A1, then add:		
Reagent 2	200 µL	200 µL
Mix and read absorbance A2 within 5 - 60 min.		

ΔA = (A2 – A1) Sample / Standard

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Sample start

	Blank	Sample/ Standard
Mono reagent	1000 µL	1000 µL
Dist. water	10 µL	-
Sample / Standard	-	10 µL

Mix and incubate for 5 min. Read absorbance against reagent blank within 60 min.

ΔA = A Sample/Standard

Calculation

With standard or calibrator

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

Conversion factor

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

Calibrators and Controls

For the calibration of automated photometric systems the DiaSystem UniCal CC is recommended. The assigned values of calibrators have been made traceable to a primary phosphorus standard (traceable to the reference material NIST-SRM 723). For internal quality control DiaSystem UniLab N and DiaSystem UniLab P or DiaSystem UniLab Urine should be assayed with each batch of samples. Each laboratory should establish corrective action in case of deviations in control recovery.

	Cat.-No.	Kit size
UniCal CC	06 00122 70 04 0018	6 x 3 mL
UniLab N	07 00123 70 05 0030	6 x 5 mL
UniLab P	07 00124 70 05 0030	6 x 5 mL
UniLab Urine Level 1	07 00125 70 04 0030	6 x 5 mL
UniLab Urine Level 2	07 00126 70 04 0030	6 x 5 mL

Performance Characteristics

All concentrations given in mg/dL refer to phosphorus.

Measuring range

The test has been developed to determine phosphorus concentrations within a measuring range from 0.2 – 30 mg/dL (0.065 – 9.69 mmol/L). When values exceed this range samples should be diluted 1 + 10 with NaCl solution (9 g/L) and the result multiplied by 11.

Specificity/Interferences

No interference was observed by ascorbic acid up to 30 mg/dL, bilirubin up to 60 mg/dL, hemoglobin up to 1000 mg/dL and lipemia up to 2000 mg/dL triglycerides. Please be aware that ditaurobilirubin interferes from the concentration 3 mg/dL on, when phosphate is measured on systems which are unable to handle a second wavelength. For further information on interfering substances refer to Young DS [6].

Sensitivity/Limit of Detection

The lower limit of detection is 0.2 mg/dL (0.065 mmol/L).

Precision (at 37 °C)

Intra-assay n = 20	Mean [mg/dL]	SD [mg/dL]	CV [%]
Sample 1	2.02	0.033	1.61
Sample 2	3.90	0.044	1.12
Sample 3	5.82	0.050	0.86

Inter-assay n = 20	Mean [mg/dL]	SD [mg/dL]	CV [%]
Sample 1	2.12	0.047	2.22
Sample 2	4.66	0.061	1.31
Sample 3	5.91	0.064	1.07

Method Comparison

A comparison of DiaSystem Phosphorous (y) with a commercially available test (x) using 75 samples gave following results:
y = 1.016 x – 0.150 mg/dL; r= 1.000.

Reference Range

Serum/Plasma [1]

	Phosphorus	
	[mg/dL]	[mmol/L]
Adults	2.6 – 4.5	0.84 – 1.45
Children / Adolescents:		
1 – 30 days	3.9 – 7.7	1.25 – 2.50
1 – 12 month(s)	3.5 – 6.6	1.15 – 2.15
1 – 3 years	3.1 – 6.0	1.00 – 1.95
4 – 6 years	3.3 – 5.6	1.05 – 1.80
7 – 9 years	3.0 – 5.4	0.95 – 1.75
10 – 12 years	3.2 – 5.7	1.05 – 1.85
13 – 15 years	2.9 – 5.1	0.95 – 1.65
16 – 18 years	2.7 – 4.9	0.85 – 1.60

Plasma [3]

Concentrations of inorganic phosphate are about 0.2 to 0.3 mg/dL (0.06 to 0.10 mmol/L) lower in heparinized plasma than in serum.

Urine [3]

0.4 – 1.3 g/24 h (12.9 – 42.0 mmol/24 h)

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

Literature

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5. Guder WG, Zatwa B et al. The quality of Diagnostic Samples. 1st ed. Darmstadt: Git Verlag, 2001; p. 40-1, 52-3.
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7. Bakker AJ, Mücke M. Gammopathy interference in clinical chemistry assays: mechanisms, detection and prevention. Clin Chem Lab Med 2007; 45(9): 1240-1243.

Manufacturer

DiaSystem Scandinavia AB
Datorgatan 3, Sweden – 561 33 Jönköping
Phone +46 36 126220 ▪ Fax +46 36 187730
info@diasystem.se ▪ www.diasystem.se

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