

Phosphorus, Inorganic

REF: 294 001 (4 x 25 ml) 100 test
 REF: 294 002 (2 x 100 ml) 200 test
 REF: 294 003 (4 x 100 ml) 400 test

Intended Use

Spectrum Diagnostics phosphorus reagent is intended for the in-vitro quantitative, diagnostic determination of phosphorus in human serum and urine on both automated and manual systems.

Background

The body of human adult contains approximately 620 g of phosphorus entirely in the form of phosphates distributed fairly equally between extracellular and intracellular compartments. About 85 % of extracellular phosphate occurs in inorganic forms as hydroxyapatite. In plasma or serum, most phosphate exist in the inorganic form (Pi); this fraction is present as the mono-and dihydrogen forms, the relative proportions varying with the pH. Intracellularly, phosphate occurs mainly as phospholipids and phosphoproteins; this fraction is termed organic phosphate. Increased serum phosphate levels may occur in renal failure, hypervitaminosis D, and hypoparathyroidism. Reduced serum phosphate levels are seen in vitamin D deficiency, rickets, hyperparathyroidism, and fanconi's syndrome.

Method

UV – phosphomolybdate method.

Assay principle

Inorganic phosphate reacts with ammonium molybdate in presence of sulfuric acid to form nonreduced phosphomolybdate.



the concentration of phosphomolybdate formed is directly proportional to the inorganic phosphate concentration. It is determined by measuring the increase in absorbance at 340 nm.

Reagents

Standard phosphorus (ST)	
5 mg/dl	1. 61 mmol/L
Reagent (R)	
ammonium molybdate	3.5 mmol/L
sulphuric acid	750 mmol/L
Surfactants	1 %

(C)-Corrosive contains caustic materials.

R35 Causes severe burns.

R41 Risk of serious damage to eyes.

S26 In case of contact with eyes, rinse immediately with plenty of water and seek medical advice.

S28 After contact with skin, wash immediately with plenty of soap and water.

For further information, refer to the Phosphorus, Inorganic reagent material safety data sheet.

Precautions and Warnings

Do not ingest or inhale. In case of contact with eyes or skin; rinse immediately with plenty of soap and water. In case of severe injuries; seek medical advice immediately.











Reagent Storage and Stability

Reagents are supplied ready-to-use and stable until expiration date stated on label when stored refrigerated at 2 - 8 °C

Deterioration

If absorbance of reagent measured at 340 nm against distilled water as reference is greater than 0.5, it should be discarded.

SYMBOLS IN PRODUCT LABELLING

	Authorized Representative		Use by/Expiration Date
	For in-vitro diagnostic use		CAUTION. Consult instructions for use
	Batch Code/Lot number		Manufactured by
	Catalogue Number		(C) - Corrosive
	Consult instructions for use		Temperature Limitation

Specimen Collection and Preservation

Serum and plasma

Serum specimens collected from fasting patients are the preferable specimens since meals lower inorganic phosphate level. The heparin is the only acceptable anticoagulant. EDTA, fluoride and citrate lower the inorganic phosphate level. Serum and plasma should be separated from erythrocytes as soon as possible to avoid the leakage of inorganic phosphate and phosphate esters into the plasma media. The biological half-life in serum is few minutes.

Stability: 1 day at 15 – 25 °C ; 4 days at 4 – 8 °C;
 1 year at -20 °C

Urine

Urine specimens should be collected in an acid-washed, detergent-free container. Acidify with hydrochloric acid after collection (pH<3).
Stability : 2 days at 15 – 25 °C ; 6 months at 2 – 8 °C;
 Urine samples should be diluted 1 : 10 (1 + 9) with distilled water before assay ; multiply the result by 10.

System Parameters

Wavelength	340 nm
Optical path	1 cm
Assay type	End-point
Direction	Increase
Sample : Reagent Ratio	1 : 100
e.g.: Reagent volume	1 ml
Sample volume	10 µl
Temperature	15 – 25 °C or 37 °C
Zero adjustment	Reagent blank
Incubation time	10 minutes at 15 – 25 °C or 5 minutes at 37 °C
Reagent Blank Limits	Low 0.00 AU High 0.5 AU
Sensitivity	1 mg/dL
Linearity	20 mg/dL (6.4 mmol/L)

Procedure

	Blank	Standard	Sample
Reagent	1 ml	1 ml	1 ml
Distilled water	10 µl	-----	-----
Standard	-----	10 µl	-----
Sample	-----	-----	10 µl

Mix, wait for 10 minutes at 15 – 25 °C or 5 minutes at 37 °C, then measure absorbance of specimen (A_{specimen}) and standard (A_{standard}) against reagent blank within 30 minutes.

Calculation

$$\text{Serum phosphorus conc. (mg/dl)} = \frac{A_{\text{specimen}}}{A_{\text{standard}}} \times 5$$

$$\text{Urine phosphorus conc. (mg/dl)} = \frac{A_{\text{specimen}}}{A_{\text{standard}}} \times 5 \times 10$$

Note: For turbid highly icteric sera, prepare a serum blank by adding 10 ml serum to 1 ml saline into a labeled test tube. Read absorbance of serum blank at 340 nm vs water and subtract from test absorbance before calculating results.

Quality Control

Normal & abnormal commercial control serum of known concentrations should be analyzed with each run.

Performance Characteristics

Precision

Within run (Repeatability)

	Level 1	Level 2
n	20	20
Mean (mg/dL)	5.49	8.92
SD	0.21	0.128
CV%	3.83	1.43

Run to run (Reproducibility)

	Level 1	Level 2
n	20	20
Mean (mg/dL)	5.61	9.1
SD	0.29	0.133
CV%	3.97	1.5

Methods Comparison

A comparison between Spectrum Diagnostics Phosphorus reagent and a commercial reagent of the same methodology was performed on 20 human sera. A correlation of 0.947 was obtained.

Sensitivity

When run as recommended, the minimum detection limit of this assay is 1 mg/dL.

Linearity

The reaction is linear up to phosphorus concentration of 20 mg/dL. Specimens showing higher concentration should be diluted 1+4 using physiological saline and repeat the assay (result × 5).

Interfering Substances (Serum, plasma)

Haemolysis

Avoid haemolysis since RBCs contain very high levels of inorganic phosphate .

Icterus

No significant interference up to a bilirubin level of 30 mg/dL.

Lipemia

No significant interference.

Anticoagulants

EDTA, citrate and fluoride interfere with the test .

Expected Values

Serum (fasting)		
Adults	: 2.7 – 4.5 mg/dL	(0.87 – 1.45 mmol/L)
Children < 12 years	: 4.5 – 5.5 mg/dL	(1.45 – 1.78 mmol/L)
Children < 1 year	: 4.5 – 6.7 mg/dL	(1.45 – 2.16 mmol/L)
Neonates	: 5.0 – 9.6 mg/dL	(1.60 – 3.10 mmol/L)
Urine (24 hrs)	: 0.3 – 1.0 g/24 hrs	(11 – 32 mmol / day)

Spectrum Diagnostics does not interpret the results of a clinical laboratory procedure; interpretation of the results is considered the responsibility of qualified medical personnel. All indications of clinical significance are supported by literature references.

Analytical Range

1 – 20 mg/dL.

Waste Disposal

This product is made to be used in professional laboratories. Please consult local regulations for a correct waste disposal.

S56: dispose of this material and its container at hazardous or special waste collection point.

S57: use appropriate container to avoid environmental contamination.

S61: avoid release in environment. refer to special instructions/safety data sheets.

References

1. Daly JA, Ertingshausen G: Direct method for determination of inorganic phosphate in serum with the centerfichem. Clin Chem 18:263, 1972.
2. Frankel S:Electrolytes. In: Gradwhol's clinical laboratory methods and diagnosis, 6 th ed. S Frankel, S Reitman, Editors, Mosby, St. louis (MO), 1963, p 188, 1963 .
3. Hanok A, Kao J: The stability of a reconstituted serum for the assay of fifteen chemical constituents. Clin Chem 14:58, 1968 .
4. young DS: Effects of drugs on clinical laboratory tests. 3 ed ed., AACC press, Washington (DC), 1990; Supplement No. 1, 1991 .

ORDERING INFORMATION

CATALOG NO.	QUANTITY
294 001	4 x 25 ml
294 002	2 x 100 ml
294 003	4 x 100 ml



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