

# PHOSPHORUS- Single Reagent

Diagnostic reagent for the in-vitro quantitative determination of Phosphorus in human serum, plasma and urine on both manual and automated systems.

REF: V/PH02.025	50 test	REF: V/PH02.050	100 test
REF: V/PH04.025	100 test	REF: V/PH02.100	200 test

## CLINICAL SIGNIFICANCE

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 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 PRINCIPLE

UV – phosphomolybdate method.  
Inorganic phosphate reacts with ammonium molybdate in presence of sulfuric acid to form non-reduced phosphomolybdate.

Phosphate + Ammonium molybdate  $\xrightarrow{\text{H}_2\text{SO}_4}$  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.

## REAGENT COMPOSITION

<b>R1: Standard</b>	5 mg/dl (1.61 mmol/L)
<b>R2: Reagent</b>	
ammonium molybdate	3.5 mol/L
sulphuric acid	750 mmol/L
Surfactants	1 %

## PRECAUTIONS AND WARNINGS

Reagent to be handled by entitled and professionally educated person.

Good Laboratories practices using appropriate precautions should be followed in:

- Wearing personnel protective equipment (overall, gloves, glasses).
- Do not pipette by mouth.
- 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.
- Respect country requirement for 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.

For further information, refer to the **Lab.Vie** Phosphorus reagent material safety data sheet.

## REAGENT PREPARATION, STORAGE AND STABILITY

**Lab.Vie** Phosphorus reagents are supplied ready-to-use. All reagents are stable until expiration date stated on label when stored at 2-8°C.

### Deterioration

The **Lab.Vie** Phosphorus reagent is normally clear, do not use reagent if it is turbid.

## SPECIMEN COLLECTION AND PRESERVATION

### Serum or plasma

- Fresh serum collected in the fasting state is the preferred specimen since serum inorganic phosphate levels are lower after meals.
- Use of anticoagulants other than heparin may interfere with the formation of the phosphomolybdate complex and should not be used.
- Serum or plasma should be separated from blood cells as soon as possible, to avoid the leakage of inorganic phosphorus and phosphate esters into the plasma media.
- Inorganic phosphorus is stable 7 days refrigerated at 40°C and for three weeks when frozen.

### Urine

- Urine specimens should be collected in acid-washed bottles.
- 24 hours specimens should be collected in containers containing 5 ml of 6.0 mol/l HCl
- Inorganic phosphorus in acidified urine specimens is stable if stored at room temperature, refrigerated or frozen.
- Stored urine specimen must be mixed well and diluted 1:10 in distilled water prior analysis.

## 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
Incubation time	10 min at 15-25°C 5 min at 37°C
Zero adjustment	Reagent blank
Sensitivity	1 mg/dL
Linearity	20 mg/dL

## EQUIPMENT REQUIRED NOT PROVIDED

- Sterile Syringe
- Analytical tubes, automatic pipet
- Centrifuge and spectrophotometer

## ASSAY PROCEDURE

	Blank	Standard	Specimen
Reagent	1.0 ml	1.0 ml	1.0 ml
Standard		10 µl	
Specimen			10 µl

Mix, incubate for 5 minutes at 37°C, or 10 minutes at 15-25°C. Measure absorbance of specimen and standard against reagent blank. The color is stable for at least 30 minutes.

## CALCULATION

Phosphorus concentration (mg/dl) =  $\frac{\text{Abs. specimen} \times 5}{\text{Abs. standard}}$

## QUALITY CONTROL

To ensure adequate quality control, it is recommended that normal and abnormal commercial control serum of known concentrations included in each run. If control values are found outside the defined range, check the instrument calibration, and reagent for problems. If control still out of range please contact **Lab.Vie.** technical support.

## PERFORMANCE CHARACTERISTICS

<b>Precision</b>	Within run (Repeatability)		Run to run (Reproducibility)	
	Normal level	High level	Normal level	High level
n	20	20	20	20
Mean mg/dl	4.09	7.12	4.11	7.09
SD.	0.03	0.046	0.09	0.06
CV. %	0.62	0.80	2.15	0.80

The results of the performance characteristics depend on the analyzer used.

### Accuracy (Methods Comparison)

Result obtained from **BioScien** Phosphorus reagent compared with commercial reagent of the same methodology performed on 20 human sera give a correlation of 0.857.

### Sensitivity

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

### Linearity

The reaction is linear up to concentration of 20 mg/dl. Specimens showing higher concentration should be diluted 1+1 using physiological saline and repeat the assay (result×2).

## INTERFERING SUBSTANCES

### 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











	mg/dl	mmol/l
<b>Serum / Plasma</b>		
Adults	2.7 – 4.5	0.87 – 1.45
Children <12 years	4.5 – 5.5	1.45 – 1.78
Children <1 years	4.5 – 6.7	1.45 – 2.16
Neonates	5 – 9.6	1.60 – 3.10
Urine (24 hrs.)	0.3 – 1 g/hrs.	11 – 32 mmol/day

## DYNAMIC RANGE

1.0– 20 mg/dl.

## 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: Gradwohl'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.

SYMBOLS IN PRODUCT LABELLING		
	For in-vitro diagnostic use	 Number of <n> test in the pack
	Batch Code/Lot number	 Caution
	Catalogue Number	 Do not use if package is damaged
	Temperature Limitation	 Consult Instruction for use
	Expiration Date	
	Manufactured by	