

Quantitative determination of Phosphorus by means of Molybdate U. V. Test.

Only for in vitro use in clinical laboratory (IVD).

Intended Use: Phosphorus(Inorganic) test reagent/kits is a medical device intended for the estimation of Phosphorus(Inorganic) in serum, plasma or urine.

Clinical Significance

Phosphorus is an essential mineral for tissue bone formation and is required by every cell in the body for normal function. Approximately 85% of the body phosphorus is found in bone and in teeth. Low levels of phosphorus can be caused by hyper vitaminosis D, primary hyper parathyroidism, renal tubular disorders, antacids or malabsorption. High levels of phosphorus can be caused by diet, bone metastases, liver disease, alcohol ingestion, diarrhea and vomiting.

Principle

When Inorganic phosphorus reacts with ammonium molybdic, in an acidic medium, it form Phosphomolybdate complex, which is measured in the U.V. range i.e. 340nm. The absorbance of this complex is directly proportional to the amount of inorganic phosphorus present in the sample.

Reagents

Reagent 1: Molybdate Reagent : 50 x 1 mL
Reagent 2: Phosphorus Standard (05mg/dL) : 1 x 2 mL
Prewashed yellow Micropipette tips : 50 nos.

Storage Instructions and Reagent Stability

The reagent is stable up to the end of the indicated month of expiry, if stored at 2-8 °C, protected from light and contamination is avoided. Do not freeze the reagents

Reagent Preparation & Stability

Reagent 1 is ready for use.

Specimen and Stability

Serum or plasma: Free of hemolysis, should be removed from the clot as quickly as possible to avoid elevation of serum phosphorus from hydrolysis or leakage of phosphate present in erythrocytes.

Urine: acidify the urine with few drops of concentrated HCl and dilute the sample 1/10 with distilled water. Mix. Multiply the result by 10 (dilution factor).

Stability in serum /plasma : 7 days at 2 – 8 °C
Urine : 10 days at 2 – 8 °C

Additional Equipment

- Spectrophotometer or colorimeter measuring at 340 nm.
- Matched Cuvette 1.0 cm light path.
- General laboratory equipment

Assay Procedure

- Assay conditions:
Wavelength: 340 nm
Cuvette: 1 cm. light path
Temperature 37 / 30 / 25°C

- Adjust the instrument to zero with distilled water

- Pipette into a Cuvette

Dispense	Blank	Standard	Samples
Reagent	1000 µL	1000 µL	1000 µL
Distilled Water	10 µL		-
Sample	-	-	10 µL
Standard	-	10 µL	-

- Mix, incubate for 5 min. at Room Temperature.

- Read absorbance against Reagent blank.

Calculation

$$\text{Phosphorus in mg/dL} = \frac{(\text{Abs. T}) - (\text{Abs. B})}{(\text{Abs. S}) - (\text{Abs. B})} \times 5 (\text{Standard})$$

Application sheets for automated systems are available on request.

Calibrators and Controls

It is recommended that appropriate quality control sera and/or controls be run with each assay batch to monitor procedural parameters.

Reference Range

Serum or plasma	
Children	4.0 – 6.50 mg/dL
Adults	2.5 – 5.0 mg/dL
Urine	
Adults	0.3 – 1.0 gm /24 hours

These values are for orientation purpose; each laboratory should establish its own reference range.

Performance Characteristics

Linearity: Up to 20 mg/dL

When values exceed this range the samples should be diluted appropriately with Distilled water and the result multiplied by the dilution factor.

Mode	End point
Wavelength (nm)	340 nm
Sample Volume (µl)	10 µL
Working Reagent Volume(µl)	1000 µL
Lag time (Sec.)	5 sec
Standard(mg/dl)	5 mg/dl
Incubation Time (sec)	5 minutes
Reaction Temperature (°C)	R.T.
Reaction Direction	Increasing
Linearity (mg/dl)	20 mg/dl
Blank with	Reagent
Units	mg/dl

Literature

- Goodwin. J.F. (1970) Clin Chem16919) : 776

For in vitro Diagnostic use only.