

# Phosphorus Estimation Kit High-Q Phosphorus-ML

Molybdate -UV/Endpoint Method

## Intended Use:

Kit for the quantitative determination of phosphorus in human serum.

## Summary and Clinical Significance :

88% of the phosphorus contained in the body is localized in bone in the form of calcium phosphate as the apatite  $Ca_2+[Ca_3(PO)_2]_2-3$ . The remainder is involved in intermediary carbohydrate metabolism and in physiologically important substances such as phospholipids, nucleic acids and ATP. Phosphorus occurs in blood in the form of inorganic phosphate and in organically bound phosphoric acid. The small amount of extracellular organic phosphorus is found almost exclusively in the form of phospholipids. The ratio of phosphate to calcium in the blood is approximately 6:10. An increase in the level of phosphorus causes a decrease in the calcium level. The mechanism is influenced by interactions between parathormone and vitamin D. Hypoparathyroidism, vitamin D intoxication and renal failure with decreased glomerular phosphate filtration give rise to hyperphosphatemia. Hypophosphatemia occurs in rickets, hyperparathyroidism and Fanconi's syndrome. The preferred method for the determination of inorganic phosphorus is based on the formation of ammonium phosphomolybdate with subsequent reduction to molybdenum blue. Reagent stability problems often occur with this method. The method presented here is based on the reaction of phosphate with ammonium molybdate to form ammonium phosphomolybdate without reduction. The addition of an accelerator gives rise to a more rapid rate of reaction and the application of sample blanking yields more precise results.

## Test principle:

Inorganic phosphate forms an ammonium phosphomolybdate complex having the formula  $(NH_4)_3[PO_4(MoO_3)_2]_{12}$  with ammonium molybdate in the presence of sulfuric acid. The complex is determined photometrically in the ultraviolet region (340 nm).

## Reagent Concentration:

- Phosphorus Reagent  
H<sub>2</sub>SO<sub>4</sub> : 280 mmol/l  
NaCl : 154 mmol/l  
Detergent : 2%
- Phosphorus Standard  
Inorganic phosphorus 5 mg/dl.

## Preparation and stability:

All the reagents are ready to use and they are stable up to the expiry date indicated on the labels when they are properly stored at when stored 4°C.

## Specimen:

Serum is the preferred specimen.

Plasma should not be used as anticoagulants may cause false low results.

## Limitations - interference:

No significant interference was observed from Bilirubin up to 92 mg/dl, hemoglobin up to 1100 mg/dl, and triglycerides up to 1650 mg/dl

## Procedure:

Pipette into test tubes labeled Blank (B), Standard (S) and Test (T) as follows:

Reagent	Blank (B)	Standard (S)	Test (T)
Phosphorus Reagent	1.0 ml	1.0 ml	1.0 ml
Phosphorus Standard (Conc : 5 mg/dl)	-	10 µl	-
Specimen	-	-	10 µl

## Mix and incubate for 5minutes at 37°C.

Mix and read absorbance of Standard (S) and Test (T) against Blank (B) at 340 nm.

## Calculations:

$$\text{Phosphorus in mg/dl} = \frac{\text{Abs. of T}}{\text{Abs. of S}} \times 5$$

## System Parameters:

Reaction type	:	End Point
Wave length	:	340
Flow cell Temp.	:	37°C
Sample volume	:	10µl
Reagent volume	:	1000µl
Standard concentration	:	5
Units	:	mg/dl
Blanking with	:	Reagent
Low normal	:	2.4 (Adults)
High normal	:	5.0 (Adults)
Linearity	:	20

## Quality Control:

To ensure adequate quality control, the use of commercial reference control serum is recommended with each assay batch. Use of Quality Control material checks both, the instrument and the reagent functions.

## Measuring Range:

0.3 - 20 mg/dl

Samples containing higher concentrations of phosphorus more than 20 mg/dl should be diluted manually with 0.9% NaCl or distilled or deionized water (e.g. 1:4). Multiply the result by the appropriate dilution factor (e.g. 4).

## Expected values:

Adults	:	2.4 - 5.0 mg/dl
Children	:	4.0 - 7.0 mg/dl

## Inorganic Phosphorus

### Analytical sensitivity (lower detection limit)

0.3 mg/dl. The lower detection limit represents the lowest measurable phosphorus concentration that can be distinguished from zero.

## Imprecision:

Reproducibility was determined using controls in an internal protocol. The following results were obtained:

Within run			
Sample	Mean mmol/l	SD mmol/l	CV%
Control serum 1	1.17	0.011	0.94
Control serum 2	1.59	0.012	0.75
Control serum 3	2.08	0.019	0.91
Between Day			
Sample	Mean mmol/l	SD mmol/l	CV%
Control serum 1	1.31	0.025	1.91
Control serum 2	1.68	0.034	2.02
Control serum 3	1.90	0.022	1.16

## Method comparison:

A comparison of High-Q Phosphorus - ML (y) with a commercial obtainable assay (x) gave the following result:  $y = 0.991x + 0.007$ ;  $r = 0.996$

## Notes

- Discard the phosphorus reagent if the absorbance of the same is more than 0.300 against distilled water at 340 nm.
- If the phosphorus value exceeds linearity limit then dilute the specimen suitably with normal saline and repeat the assay. In such case the assay value should be multiplied by the dilution factor to obtain correct phosphorus value of the specimen.
- Strong lipemic and haemolytic sera not be used.
- Contaminated glassware is the greatest source of error. Disposable plastic tubes and clean glassware are recommended for the test.

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- The reagent contains sulphuric acid. Avoid contact with the skin and mucous membrane. If you come in contact with the reagent wash thoroughly with water.
- Do not use standard in multiples to check the linearity as it may lead to precipitation of the reagent because Standard and reagent contain sulphuric acid. Linearity should be checked with serum based controls/calibrators containing high phosphorus values.

#### Literature:

- Bablok W. et al. A General Regression Procedure for Method Transformation. J Clin Chem Clin Biochem 1988;26:783-790.
- Burtis C.A., Ashwood E.R.,(ed). Tietz Textbook of Clinical Chemistry, 2nd ed. Philadelphia, PA: WB Saunders, 1994:1909.
- Fiske C.H., Subbarow Y. The colorimetric determination of phosphorus. J Biol Chem 1925;66:375-400.

#### Ordering information

Ref./Cat. No.	Pack Size	Presentation
P-PHO - 50	2 x 25 ml	Mono Reagent
















### Product Features

- Liquid Stable, Ready to use Mono Reagent
- 5 Minutes single step End Point Reaction
- Aqueous Phosphorus standard provided (Standard Conc: 5 mg/dl)
- Superior over Phenyl hydrazine and Amino naphtho sulphonic acid methods
- With Lipid Clearing Factor (LCF).
- Linearity :20 mg/dl
- Measuring Wavelength: 340 nm
- Serum is the only specimen
- Available as Multi Purpose Reagents and Dedicated System Packs



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#### Symbols used with IVD devices

	Date of manufacture		Manufactured by
	In vitro diagnostic device		Keep away from sunlight
	Do not freeze		This way up
	Use by (yyyy-mm-dd or mm/yyyy)		Reagent
	Calibrator Material		Batch code
	Temperature limitation (store at)		Control
	Consult instructions for use		Keep dry Keep away from rain
	Catalog Number		

eIFU Indicator



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