

LiquiMAX Phosphorus-SLR

Molybdate -UV/Endpoint Method

ORDERING INFORMATION

Ref. No.	Pack Size	Presentation
AVPHO - 50	50 x 1 ml	Mono Reagent
AVPHO - 100	4 x 25 ml	Mono Reagent

INTENDED USE:

LiquiMAX Phosphorus-SLR is an in-vitro diagnostic kit is use for the quantitative determination of phosphorus in human serum.

PRODUCT FEATURES

1. Liquid Stable, Ready to use Mono Reagent
2. 5 Minutes single step End Point Reaction
3. Aqueous Phosphorus standard provided (Standard Conc: 5 mg/dl)
4. Superior over Phenyl hydrazine and Amino naphtho sulphonic acid methods
5. With Lipid Clearing Factor (LCF).
6. Linearity :20 mg/dl
7. Measuring Wavelength: 340 nm
8. Serum is the only specimen
9. Available as Multi Purpose Reagents and Dedicated System Packs

CLINICAL SIGNIFICANCE :

88% of the phosphorous contained in the body is localized in bone in the form of calcium phosphate as the apatite $Ca_2+[Ca_3 (PO)_2]_3$. 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.

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).

STORAGE 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.

KIT COMPONENTS

1. Phosphorus Reagent
2. Phosphorus Standard : Concentration as stated on the label

COMPOSITIONS

1. Phosphorus Reagent
 - H₂SO₄ : 280 mmol/l
 - NaCl : 154 mmol/l
 - Detergent : 2%
2. Phosphorus Standard
 - Inorganic phosphorus 5 mg/dl.

REAGENT RECONSTITUTION & STABILITY

Reagent are liquid stable no need for reconstitution.

When the reagent is stored properly at 2-8°C & the contamination avoided, it is stable up to the expiry date mention on the label & kit box.

MATERIAL REQUIRED BUT NOT PROVIDED

Laboratory Instrumentation, Spectrophotometer UV/VIS with thermostatic cuvette holder or clinical chemistry analyzer: semi auto, calibrated micropipettes, glass or high quality polystyrene cuvettes, test tube/rack, heating bath controls, saline.

REAGENT DETERIORATION

Discard any turbid reagent if blank reagent absorbance exceeds 0.3 at 340 nm against distilled water.

WARNING & PRECAUTIONS

- Reagent may contain some non reactive and preservative components. It is recommended to handle carefully, avoiding contact with skin and ingestion.
- Specimen should be considered infectious and handled appropriately.
- Contamination by soap or glycerol will affect this assay.
- Perform the test according to the general " Good Laboratory Practice" GLP

SPECIMEN COLLECTION AND STORAGE

Serum is the preferred specimen.

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

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

TEST 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

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

EXPECTED VALUES:

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

QUALITY CONTROL & CALIBRATION

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.

PERFORMANCE CHARACTERISTICS

1. Linearity

Linearity : 20 mg/dl

2. Sensitivity/ Limit of Detection (LOD)

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

3. Interferences

Bilirubin (mixed isomers): Less than 10% interference up to 600µmol/l
Bilirubin. Haemolysis: Less than 10% interference up to 1.25 g/l Haemoglobin.
Lipemia: Less than 10% interference up to 2.5 g/l Intralipid.

4. Precision:

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

Intra-Assay

Sample	Mean (mg/dl)	SD (mg/dl)	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

Inter-Assay

Sample	Mean (mg/dl)	SD (mg/dl)	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

5. Method comparison:

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

LIMITATIONS:

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).

WASTE DISPOSAL

Reagents must be disposed off in accordance with local regulations.














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.
- 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.

REFERENCES

- 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.

Symbols Used on Pack

 REF	Catalogue Number		Warning/Caution
 LOT	Batch No.		In vitro diagnostic device
	Manufacturing Date		Storage Limit
	Expiry Date		Consult instruction for use
	Manufacturer		Keep away from sunlight
	Keep Dry		Do not use if package is damaged
	Contains sufficient no. of test		



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