

PHOSPHORUS (Ammonium Molybdate Method-UV,End Point)

Intended Use

The reagents are used for the quantitative determination of Phosphorus in serum, plasma or urine. For in-vitro diagnostic use only.

Introduction

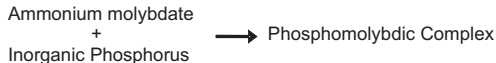
Total body phosphorus in a healthy person is about 700 to 800mg; 85 % of which is present in the skeleton in hydroxyapatite phase, the remaining 15 % is in the soft tissues. A serum Inorganic phosphate reflects only a very minor percentage of total body phosphorus; however it is easily measurable and gives a clue of the status of body phosphorus stores. In serum inorganic phosphate exists in two forms: dihydrogen phosphate ($H_2PO_4^-$) and monohydrogen phosphate (HPO_4^{2-}). The majority of phosphate in the body is in the organic form as a complex with carbohydrates, proteins and lipids. Increased serum phosphorus levels are observed in the hyperthyroidism, hypervitaminosis, renal failure and neoplastic disease such as leukemias, lymphomas. Decreased levels of phosphorus level are observed in the vitamin-D deficiency, hyperparathyroidism and mal-absorption etc.

Method

Ammonium Molybdate method-UV,End Point.

Principle

Inorganic phosphorus reacts with ammonium molybdate in an acidic medium to form a phosphomolybdate complex which absorbs light at 340nm. The absorbance at this wavelength is directly proportional to the amount of inorganic phosphorus present in the sample.



Reagent Composition

Reagent 1:

Sulphuric Acid	208 mmol/l
Ammonium molybdate	0.4 mmol/l

Reagent 2:

Phosphorous Standard	5 mg/dl (1.61 mmol/l)
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Precautions

Following precautions should be taken:

- All the glassware should be washed with 0.1 M HCl (Washing solution) and properly rinsed with distilled water and dried before use.
- Avoid ingestion, do not pipette by mouth.
- Avoid contact with skin and eyes. If spilled, thoroughly wash affected area with water.
- Flush with plenty of water while disposing.

Reagent Storage and Stability

Unopened Reagents 1 are stable till expiry mentioned on the label when stored at 2-8°C.

Standard Reagent 2 is stable till expiry as mentioned on the label when stored at 2-8°C.

Note: On request, Reagent 3 (Phosphorus: 10 mg/dl) & Reagent 4 (Phosphorus: 15 mg/dl) can be provided for linearity check with Reagent 2 (Phosphorus: 5 mg/dl - Standard) .

Reagent Preparation

Reagents are ready for use.

Reagent Deterioration

Reagent should be clear. Turbidity and/or precipitation may be because of reagent deterioration.

Sample Collection and Storage

Unhaemolysed serum or heparinised plasma or urine can be used for the testing. Fluoride is not recommended to be used as anti-coagulant as it may result in low results. It is recommended to use freshly collected samples for assay. Separated plasma samples can be stored for 7 days at 2-8°C.

General Assay Parameters

Mode	End Point
Wavelength 1 (nm)	340
Wavelength 2 (nm)	-
Blank with	Reagent
Sample Volume (µl)	5/10
Reagent R1 (µl)	500/1000
Incubation Time (min.)	5
Incubation Temperature(°C)	37°C
Normal Low (mg/dl)	2.5
Normal High (mg/dl)	4.8
Linearity (mg/dl)	Upto 15
Standard Conc. (mg/dl)	5
Units	mg/dl

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Procedure

Always use 0.1 M HCl washed & dried test tubes before test the assay. One reagent blank and one standard are sufficient for each assay series.

Pipette into test tubes:

Particulars	Blank	Standard	Sample
Reagent 1	1000µl	1000µl	1000µl
Dist. Water	10µl	-	-
Reagent 2	-	10µl	-
Sample	-	-	10µl

Mix well & incubate for 5 min at 37°C. Measure the absorbance of standard (A std) and sample (A sample) against reagent blank at 340nm

Calculation

Phosphorus concentration in the sample can be calculated using the following formula:

$$\text{Phosphorus} = \frac{\text{Absorbance of Sample}}{\text{Absorbance of Standard}} \times \text{Conc. of Std. (mg/dl)}$$

Example: If the absorbance of sample is 0.200 and the absorbance of standard is 0.18. The calculation shall be:

$$\frac{0.200}{0.18} \times 5 = 5.5 \text{ mg/dl}$$

If the phosphorus concentration exceeds 15mg/dl, dilute the sample with normal saline and repeat the assay. The reportable results in this case shall be calculated by multiplying the results obtained with dilution factor.

Reference value

Serum (Adults): 2.5 – 4.8 mg/dL

Limitations

1. The reagent and sample volumes can be altered proportionately so that the sample: reagent, ratio remains same.

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2. Hemolytic and lipemic samples may result in falsely elevated results. To avoid false results sample blank may be used as mentioned below:

- Add 10µl of serum sample to 1000µl of Normal Saline and read absorbance at 340nm.
- Subtract the absorbance obtained as above, from the absorbance of test. Use this corrected absorbance for calculation.
- Phosphorus contamination must be avoided. It is highly recommended to use disposable glass or plasticware.

Quality Control

The patient results obtained for each batch can be validated by using normal and abnormal control sera with assayed values for phosphorus.

Performance

Linearity Limit: 15mg/dl

Precision:

Within run

Control	Control 1	Control 2
No. of samples	20	20
Mean (mg/dl)	3.40	7.80
S.D.	0.05	0.07
C.V. %	1.54	0.93

Between run

Control	Control 1	Control 2
No. of samples	60	60
Mean (mg/dl)	3.42	7.73
S.D.	0.05	0.06
C.V. %	1.28	0.82

References

1. Slatopolsky E. Pathophysiology of calcium, magnesium, and phosphorus metabolism. In: Klahr S, ed. The kidney and body fluids in health and disease. New York: Plenum Press, 1983;269–330. www.ncbi.nlm.nih.gov/bookshelf/br.fcgi?book=cm&part=A5577

2. Brautbar N, Kleeman CR. Hypophosphatemia and hyperphosphatemia: clinical and pathophysiologic aspects. In: Maxwell MH, Kleeman CR, Narins RG, eds. Clinical disorders of fluid and electrolyte metabolism. New York: McGraw-Hill, 1987;789–830.
3. Tiez, N.W., "Speciman Collection and Processing; Sources of Biological Variation," Textbook of Clinical Chemistry, 2nd Edition, WB.Saunders, Philadelphia, PA (1994). http://www.tecodiag.com/Admin/pdf/274_Packa%20ge%20Insert.PDF
4. Henry, R.J., et al.: Clinical Chemistry: Principles and Techniques 409 New York, Harper and Row 728 (1974) <http://www.rightchoicediag.com/files/pdf/RCC0052E-CE.pdf>

Pack Presentation

Product Code/ Catalogue No.	Pack Size*	Reagent 1	Reagent 2
KGPHS105.3.1	2x50ml	2x50ml	1x2ml











* Pack size may vary on market demand.

Revision No: (Ver: KGPHS106.3/1)

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Symbols

Following symbols are used in the labeling of KEE GAD kits:

	Catalogue No.		Batch No.
	CE Mark		Read instructions
	In Vitro Diagnostics		Storage temperature
	Expiry Date		Content
	Product Name		Manufactured By



Manufactured by:
KEE GAD Biogen Pvt. Ltd.
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