PHOSPHORUS (INORGANIC)

[Intended Use]

For the quantitative *in vitro* determination of inorganic phosphorus in human serum, plasma and urine.

[Summary and Explanation]

Most of the phosphorus in the body (80% to 85%) is calcium-bound and is found in the bones as hydroxyapatite crystals.The remainder is distributed throughout other cells of the body primarily as organic phosphorous in phospholipids and phosphoproteins. Plasma contains approximately 1% of total phosphate as inorganic phosphate, the fraction measured in routine biochemical analysis.

Serum phosphorus may increase in hypervitaminosis D, hypoparathyroidism and renal failure. Decreased serum phosphorus is seen in rickets (vitamin D deficiency), hyperparathyroidism and Fanconi syndrome.

[Principle of Assay]

Direct method for determining inorganic phosphate. Inorganic phosphate reacts in acid medium with ammonium molybdate to form a phosphomolybdate complex with yellow color. The intensity of the color formed is proportional to the inorganic phosphate concentration in the sample at 340 nm.

Ammonium molybdate + Sulphuric acid + Phosphate

→ Inorganicphosphate molybdate complex

[Reagent Composition]

Reagent 1: Sulfuric acid

Surfactants

Reagent 2: Sulfuric acid

Ammonium molybdate

Surfactants

Preservatives

[Components]

All reagents are ready to use.

	Content	Amount
R1	Buffer	60 ml
R2	Chromogen	60 ml
Standard 1	3.70 mg/dl	3 ml
Standard 2	8.00mg/dl	3 ml
Level 1	2.29 mg/dl (1.83-2.75)	3 ml
Level 2	3.72 mg/dl (2.98-4.47)	3 ml
Level 3	6.41 mg/dl (5.12-7.69)	3 ml

[Stability and Preparation of Reagents]

R1*Buffer*

Contents are ready to use, no preparation is required. Stable up to the expiry date when stored at 2-8˚C after opened.

R2C*hromogen solution*

Contents are ready to use, no preparation is required. Stable up to the expiry date when stored at 2-8˚C after opened.

CAL and QC *Standard and levels*

Contents are ready to use, no preparation is required. Stable up to the expiry date when stored at 2-8˚C after opened. Any inorganic phosphorus calibrator is applicable, however, Rel Assay calibrator and controls are recommended.

[Storage and Validity Date]

Storage: This kit should be stored at 2-8℃.

Validity date: The validity of each reagent is indicated on the bottle and on the kit box.

(This kit is stable for up to expiry date when stored at 2-8℃ after opened.)

[Safety precautions and warning]

1. For *in vitro* diagnostic use only.
2. Do not pipette by mouth.
3. Exercise the normal precautions required for handling laboratory reagents.
4. Wear disposable gloves while handling the kit reagents and wash hands thoroughly afterwards.
5. Do not use reagents beyond the expiry date.
6. The reagents must be used only for the purpose intended by suitably qualified laboratory personnel, under appropriate laboratory conditions.
7. Dispose cleaning liquid and also such used washing cloth or tissue paper with care, as they may also contain infectious agents.
8. Health and safety data sheets are available on request.

[Specimen collection and Storage]

Blood serum, heparinized plasma and urine could be used as sample.

Serum: Use serum with gel barrier or gel barrier-free glass or plastic tubes. Unhemolyzed serum is specimen of choice. Serum or plasma 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.

Serum samples are stable up to 1 week stored at 4˚C, 2 months at -20˚C and 6 months at -80˚C.

Urine: Urine samples should be collected in a utensil containing 20 mL of 6 mol/L HCl for a 24-hour sample to prevent precipitation of phosphate complexes.

Acidified urine samples are stable up to 10 days stored at 4˚C.

[Handling method and Calculation]

1. This product is designed to be used in the spectrophotometer, plate reader or automated biochemistry analyzer.

2. Could be used in various automated biochemistry analyzers, an example of operating application is shown below;

Serum and plasma application

Assay type	End Up
Wavelength (main/sub)	340/700 nm

	R1	R2
Reagent pipetting	90 µL	85 µL
Diluent (H2O)	78 µL	-

	Sample	Diluent (H_2O)	Diluted Sample
Sample volumes	3 µL	-	-

Urine application

Assay type	End Up
Wavelength (main/sub)	340/700 nm

	R1	R2
Reagent pipetting	26 µL	39 µL
Diluent (H_2O)	132 µL	-

	Sample	Diluent (H_2O)	Diluted Sample
Sample volumes	16 µL	144 µL	2.6 µL

3. R2 is added to the mixture two minutes after R1 and sample are mixed. Measurement is done at the end of five minutes.

Make sure that cuvettes of the auto-analyzer or plate reader are thoroughly cleaned before the assay to obtain accurate and sensitive results. Use Reagent Grade 1 water for the assay.

Mix, read the absorbance of sample, standards, control levels and water. Absorbance obtained from water is extracted from all samples, standard and control levels' absorbance. Extracted standard absorbance is accepted as 3.7 mg/dl and 8.0 mg/dl for standard 1 and standard 2 respectively. Calculate the factor and multiply the absorbance obtained from samples with factor to calculate the result. Level 1 is expected as 2.29 mg/dl, level 2 is expected as 3.72 mg/dl and level 3 is expected as 6.41 mg/dl.

[Reference range]

Serum (Adults):	2.3 - 4.7 mg/dl
Serum (Children):	4.0 - 7.0 mg/dl
Urine:	0.4 - 1.3g/24h

Each laboratory is recommended to establish their own reference values.

Serum samples collected after at least 8 hours of fasting must be used for the test. Levels are higher in the afternoon and evening. Serum phosphate concentrations are influenced by dietary intake and meals and increased by exercise.

[Performance Characteristics]

Serum:*Low linearity:* 0.7 mg/dl - *High linearity:* 25.3 mg/dl

Urine: *Low linearity:* 0.5 mg/dl - *High linearity:*186 mg/dl

If the results obtained were greater than linearity limit, dilute the sample 1/2 with NaCl 9 g/L and multiply the result by 2.

[Method Comparison]

A comparison of Rel Assay Inorganic Phosphorus (y) with a commercially available test (x) using 83 samples gave following results:

Regression equation: y=0,98x + 1,12

Correlation coefficient (r): 0.973

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

[Precision]

Intra-assay CV was determined with 30 replicates of 2 controls. The %CV result was 1,71 and inter-assay %CV result was 1,95.

[Interferences]

No interference was observed by ascorbic acid up to 30 mg/dL, bilirubin up to 40 mg/dL, hemoglobin up to 100/dL and triglyceride up to 600 mg/dL.

In order to obtain best results, pipettes and cuvettes used for the assay must be cleaned thoroughly. Make sure that no wash solution is left on pipettes and in cuvettes.

References

(1) TIETZ N.W. Text book of clinical chemistry, 3rd Ed. C.A. Burtis, E.R. Ashwood, W.B. Saunders 1999; p. 1406-1457.

(2) TIETZ N.W. Clinical Guide to Laboratory Test, 4th Ed., Philadelphia, PA: WB Saunders Company, 2006; p.852-855

(3) Daly, J.A., Ertingshausen, G., Clin. Chem. 1972; 18: 263.

(4) Farrell E C. Phosphorus. Kaplan A et al. Clin Chem. The C.V. Mosby Co. St Louis. Toronto. Princeton 1984; 1072-1074 and 418.

(5) Young DS. Effects of Drugs on Clinical LaboratoryTests. 5th ed. Volume 1 and 2. Washington, DC: TheAmerican Association for Clinical Chemistry Press2000.