COD 11508 170 mL
STORE AT 2-30ºC
Reagents for measurement of phosphorus concentration Only for *in vitro* use in the clinical laboratory

PHOSPHORUS

CE

PHOSPHORUS
PHOSPHOMOLYBDATE/UV

PRINCIPLE OF THE METHOD

Inorganic phosphorus in the sample reacts with molybdate in acid medium forming a phosphomolybdate complex that can be measured by spectrophotometry[1,2].

CONTENTS AND COMPOSITION

A. Reagent: 3 x 40 mL. Sulfuric acid 0.36 mol/L, sodium chloride 154 mmol/L.

DANGER: H314: Causes severe skin burns and eye damage. P280: Wear protective gloves/protective clothing/eye protection/face protection. P303+P361+P353: IF ON SKIN (or hair): Remove/Take off immediately all contaminated clothing. Rinse skin with water/shower.

B. Reagent: 1 x 50 mL. Sulfuric acid 0.36 mol/L, sodium chloride 154 mmol/L, ammonium molybdate 3.5 mmol/L.

DANGER: H314: Causes severe skin burns and eye damage. P280: Wear protective gloves/protective clothing/eye protection/face protection. P303+P361+P353: IF ON SKIN (or hair): Remove/Take off immediately all contaminated clothing. Rinse skin with water/shower.

S. Phosphorus Standard. 1 x 5 mL. Phosphorus 5 mg/dL (1.61 mmol/L). Aqueous primary standard.

For further warnings and precautions, see the product safety data sheet (SDS).

STORAGE

Store at 2-30ºC.

Reagents and Standard are stable until the expiry date shown on the label when stored tightly closed and if contaminations are prevented during their use.

Indications of deterioration:

– Reagents: Presence of particulate material, turbidity, absorbance of the blank over 0.500 at 340 nm.

– Standard: Presence of particulate material, turbidity.

REAGENT PREPARATION

Standard (S) is provided ready to use.

Working Reagent: Mix thoroughly in the proportion: 7 mL Reagent A + 3 mL Reagent B. Stable for 12 months at 15-30ºC.

ADDITIONAL EQUIPMENT

– Analyzer, spectrophotometer or photometer able to read at 340 ± 20 nm.

SAMPLES

Serum, heparinized plasma or urine collected by standard procedures.

Phosphorus in serum or plasma is stable for 7 days at 2-8ºC.

Collect 24-hour urine in a bottle containing 10 mL of 10% (v/v) hydrochloric acid. Stable for 10 days at 2-8ºC. Centrifuge or filter the sample and dilute 1/10 with distilled water before measurement.

PROCEDURE

1. Pipette into labelled test tubes: (Note 1)

	Reag.Blank	Sample Blank	Sample	Standard
Distilled Water	10 µL	—	—	—
Sample	—	10 µL	10 µL	—
Phos. Standard (S)	—	—	—	10 µL
Reagent (A)	—	1.0 mL	—	—
Working Reagent	1.0 mL	—	1.0 mL	1.0 mL

2. Mix thoroughly and let stand the tubes for 5 minutes at room temperature.
3. Read the absorbance (A) of the Sample Blanks at 340 nm against distilled water.
4. Read the absorbance (A) of the Samples and of the Standard at 340 nm against the Reagent Blank.

CALCULATIONS

The phosphorus concentration in the sample is calculated using the following general formula:

$$\frac{A_{Sample} - A_{Sample\ Blank}}{A_{Standard}} \times C_{Standard} \times \text{Sample dilution factor} = C_{Sample}$$

If the Phosphorus Standard provided has been used to calibrate (Note 2):

	Serum and plasma	Urine
$\frac{A_{Sample} - A_{Sample\ Blank}}{A_{Standard}}$	x 5 = mg/dL x 1.61 = mmol/L	x 50 = mg/dL x 16.1 = mmol/L

REFERENCE VALUES

Serum[3]: Adults: 2.5-4.5 mg/dL = 0.81-1.45 mmol/L
Children: 4.0-7.0 mg/dL = 1.29-2.26 mmol/L

Urine[3]: 0.4-1.3 g/24-h = 12.9-42 mmol/24-h

Concentrations in plasma are about 0.25 mg/dL (0.08 mmol/L) lower than in serum. These ranges are given for orientation only; each laboratory should establish its own reference ranges.

QUALITY CONTROL

It is recommended to use the Biochemistry Control Serum level I (cod. 18005, cod. 18009 and cod. 18042), level II (cod. 18007, cod. 18010 and cod. 18043) and the Biochemistry Control Urine (cod. 18054 and cod. 18066) to verify the performance of the measurement procedure.

Each laboratory should establish its own internal Quality Control scheme and procedures for corrective action if controls do not recover within the acceptable tolerances.

METROLOGICAL CHARACTERISTICS

– Detection limit: 0.13 mg/dL phosphorus = 0.042 mmol/L phosphorus.

– Linearity limit: 20 mg/dL phosphorus = 6.46 mmol/L phosphorus. For higher values dilute sample 1/2 with distilled water and repeat measurement.

– Repeatibility (within run):

Mean concentration	CV	n
4.34 mg/dL = 1.40 mmol/L	1.3 %	20
8.20 mg/dL = 2.65 mmol/L	0.7 %	20

– Reproducibility (run to run):

Mean concentration	CV	n
4.34 mg/dL = 1.40 mmol/L	2.9 %	25
8.20 mg/dL = 2.65 mmol/L	2.5 %	25

– Sensitivity: 48 mA·dL/mg = 149 mA·L/mmol

– Trueness: Results obtained with this reagent did not show systematic differences when compared with reference reagents (Note 2). Details of the comparison experiments are available on request.

– Interferences: hemoglobin (10 g/L), lipemia (triglycerides 10 g/L) and bilirubin (20 mg/dL) do not interfere. Other drugs and substances may interfere[4].

These metrological characteristics have been obtained using an analyzer. Results may vary if a different instrument or a manual procedure are used.

DIAGNOSTIC CHARACTERISTICS

Approximately 80% of the phosphorus in the human body is found in the calcium phosphate salts, which make up the inorganic substance of bone. The remainder is involved in the esterification of carbohydrate metabolism intermediaries and is also found as a component of phospholipids, phosphoproteins, nucleic acids and nucleotides.

Hypophosphatemia can be caused by shift of phosphate from extracellular to intracellular spaces, increased renal loss (renal tubular defects, hyperparathyroidism) or gastrointestinal loss (diarrhea, vomiting), and decreased intestinal absorption[3,5].

Hyperphosphatemia is usually secondary to inhability of the kidneys to excrete phosphate due to renal failure or hypoparathyroidism[3,5].

Clinical diagnosis should not be made on the findings of a single test result, but should integrate both clinical and laboratory data.

NOTES

1. These reagents may be used in several automatic analysers. Instructions for many of them are available on request.
2. Calibration with the provided aqueous standard may cause a matrix related bias, specially in some analyzers. In these cases, it is recommended to calibrate using a serum based standard (Biochemistry Calibrator, cod. 18011 and 18044).

BIBLIOGRAPHY

1. Gamst O and Try K. Determination of serum-phosphate without deproteinization by ultraviolet spectrophotometry of the phosphomolybdic acid complex. Scand J Clin Lab Invest 1980; 40: 483-486.
2. Muñoz MA, Balón M and Fernández C. Direct determination of inorganic phosphorus in serum with a single reagent. Clin Chem 1983; 29: 372-374.
3. Tietz Textbook of Clinical Chemistry and Molecular Diagnostics, 4th ed. Burtis CA, Ashwood ER, Bruns DE. WB Saunders Co, 2005.
4. Young DS. Effects of drugs on clinical laboratory tests, 5th ed. AACC Press, 2000.
5. Friedman and Young. Effects of disease on clinical laboratory tests, 4th ed. AACC Press, 2001.