# PHOSPHORUS CE

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|---|---|---|---|
| **REF** | 1148010 | Phosphorus | 3 x 50 mL |
| For *in vitro* diagnostic use only | | | |

# PHOSPHORUS

INORGANIC
*Colorimetric method*
ENDPOINT

## PRINCIPLE

Inorganic phosphate reacts with molybdic acid forming a phosphomolybdic complex. Its subsequent reduction in alkaline medium originates a blue molybdenum colour which intensity is proportional to the amount of phosphorus present in the sample.[1]

$$PO_4^{3-} + H^+ + (NH_4)_6\,Mo_7O_{24} \xrightarrow{P_i} \text{Phosphomolybdic Complex}$$

$$\text{Phosphomolybdic Complex} \xrightarrow[\text{REDUCTANT}]{pH > 10} \text{Molybdenum blue}$$

## REAGENT COMPOSITION

**R1** **Molybdate Reagent**. Ammonium molybdate 7 mmol/L, sulphuric acid 0.8 mol/L. **$X_i$ R:36/37/38**

**R2** **Reducing solution**. Hydroxylamine 0.64 mol/L. Catalyzers.

**R3** **Color developer**. Sodium hydroxide 3 mol/L. Stabilizers. **C R:35**

**CAL** **Phosphorus standard.** Phosphorus 5 mg/dL (1.6 mmol/L) Organic matrix based primary standard.

## STORAGE AND STABILITY

Store at 15-30ºC.
The reagents are stable until the expiry date stated on the label.

## REAGENT PREPARATION

**Working reagent**. Mix 1 volume of **R1** + 1 volume of **R2**. Stable for 8 hours at 2-8ºC protected from light.

## SAMPLES

Serum or heparinized plasma separated from cells as soon as possible, and urine (see Notes).
The phosphorus in serum or plasma is stable for 7 days at 2-8ºC. Freeze for longer storage.
Phosphorus in acidified samples of urine is stable for about 6 months at 2-8ºC.

## INTERFERENCES

- Effects of bilirubin (>20 mg/dL) and lipemia (triglycerides >10 g/L) do not interfere. Glucose (>600 mg/dL) does not interfiere.
- Hemoglobin (>10 g/L) interfere.
- Other drugs and substances may affect the phosphorus values.[2]

## MATERIALS REQUIRED

- Photometer or colorimeter capable of measuring absorbance at 740 ± 10 nm.
- Laboratory alarm clock.
- Pipettes to measure reagent and samples.

## PROCEDURE

1. Bring reagents and samples to room temperature.
2. Pipette into labeled test tubes:

| TUBES | Blank | Sample | Standard |
|---|---|---|---|
| Working Reagent | 1.0 mL | 1.0 mL | 1.0 mL |
| Sample | – | 50 µL | – |
| Standard | – | – | 50 µL |

3. Mix, let stand the tubes for 1 minute and then pipette:

| | | | |
|---|---|---|---|
| Developer | 0,5 mL | 0,5 mL | 0,5 mL |

4. Mix and let stand the tubes 10 minutes at room temperature.
5. Read the absorbance (A) of the sample and the standard at 740 nm against the reagent blank.

The color is stable for at least 30 minutes protected from light.

## CALCULATIONS

Serum, plasma

$$\frac{A_{Sample}}{A_{Standard}} \times C_{Standard} = \text{mg/dL phosphorus}$$

Samples with concentrations higher than 15 mg/dL (4.8 mmol/L) should be diluted 1:2 with saline and assayed again. Multiply the results by 2.

Urine

$$\frac{A_{Sample}}{A_{Standard}} \times 100 = \text{mg/24-hours phosphorus}$$

If results are to be expressed as SI units apply:
mg/dL x 0.323 = mmol/L

## REFERENCE VALUES[3]

Serum, plasma

| | |
|---|---|
| Children | 4.0-7.0 mg/dL (1.29-2.26 mmol/L) |
| Men | 2.5-4.5 mg/dL (0.81-1.45 mmol/L) |
| Women | 1.5-6.8 mg/dL (0.48-2.19 mmol/L) |

Urine

| |
|---|
| 0.4-1.0 g/24-h (12.9-32.3 mmol/24-h) |

It is recommended that each laboratory establishes its own reference range.

## QUALITY CONTROL

The use of a standard to calculate results allows to obtain an accuracy independent of the system or instrument used.
To ensure adequate quality control (QC), each run should include a set of controls (normal and abnormal) with assayed values handled as unknowns.

REF **1980005** HUMAN MULTISERA NORMAL
Borderline level of phosphorus. Assayed.

REF **1985005** HUMAN MULTISERA ABNORMAL
Elevated level of phosphorus. Assayed.

## CLINICAL SIGNIFICANCE

Phosphorus and calcium metabolism are interwined. In healthy persons, as serum calcium levels rise, those of phosphorus fall. Control of phosphorus levels is in part accomplished by regulation of renal excretion. However, fairly rapid fluctuations in serum inorganic phosphate can occur because the serum inorganic phosphate concentration is influenced by carbohydrate metabolism.

In *diabetes*, severe loss of phosphate is possible, since carbohydrate metabolism is deranged and phosphate tends to pass from the cell into extracellular fluid and then into plasma. It is then extracted and excreted by the Kidney.
Increased levels are associated with *hypoparathyroidism*, during insulin treatment of *diabetic coma*, and with chronic *nephritis* rising as renal failure progresses.

## NOTES

- Collect a 24-hor urine specimen into a plastic bottle containing 20 mL of 50% (v/v) HCl. Bring to 2 L with distilled water. Mix completely and test as described for serum.
- Most of the detergents and water softening products used in the laboratories contain chelating agents and phosphates. It is recommended to rinse glassware in diluted nitric and distilled water before using.

## ANALYTICAL PERFORMANCE

- ***Linearity.*** Up to 15 mg/dL
- ***Precision***

| mg/dL | Within-run | | | Between-run | | |
|---|---|---|---|---|---|---|
| Mean | 2.5 | 12.9 | 20.2 | 2.5 | 12.9 | 20.2 |
| SD | 0.07 | 0.3 | 0.2 | 0.08 | 0.35 | 0.41 |
| CV% | 2.8 | 2.3 | 0.75 | 3.2 | 2.7 | 1.54 |
| N | 5 | 5 | 5 | 5 | 5 | 5 |

Replicates: 5 for each level. Replicates: 5 for each level
Instrument: UVIKON 930 for 8 days.

- ***Sensitivity*.** Using a 1:30 sample/reagent at 740 nm, 1mg of phosphorus will produce a net absorbance of approximately 0.086.
- ***Correlation.*** This assay (y) was compared with a similar commercial method (x). The results were:
  N = 20 r = 0.978 y = 1.184x + 1.22

## REFERENCES


1. Drewes PA. Clin. Chim. Acta 39 : 81 (1972)
2. Young, D.S., Pestaner, L.D. and Gibberman, V. Clin. Chem. 21, Vol. 5, 10-432D (1975).
3. Tietz. N.W. Clinical Guide to Laboratory Tests, 3rd Edition. W.B. Saunders Co. Philadelphia, PA. (1995).


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