

Quantitative determination of Phosphorus in serum, plasma or urine. Phosphomolybdate, UV Endpoint.

**REF** CC1270 R1: 4x60 mL R2: 1x3 mL (standard)

## METHOD AND PRINCIPLE

In an acidic environment, ammonium molybdate reacts with inorganic phosphate, giving rise to ammonium phosphomolybdate whose absorbance, measured photometrically at 340 nm, is directly proportional to the concentration of the phosphorus present in the sample.

## CLINICAL SIGNIFICANCE

Phosphorus exists in the body almost exclusively as phosphate, mainly as inorganic substance of the bones, but also in cells in phospholipids and nucleic acids as well as in adenosine triphosphate, which is involved in the energy transfer. In plasma it is present as calcium phosphate, therefore the level of plasma phosphorus is strongly associated with that of calcium levels. Measurement of phosphorus in serum and urine is mainly performed to detect disorders of kidneys, bones and parathyroid glands. Increased concentrations are found in renal failure, hypoparathyroidism, pseudo-hyperparathyroidism and loss of calcium phosphate of bones and cells. Decreased values occur in malabsorption, hyperparathyroidism and vitamin D deficiency. Additional information can be obtained by supplementary measurement of calcium.

## REAGENT COMPOSITION

### Reagent (R1)

Ammonium molybdate	0.40 mmol/L
Sulphuric acid	210 mmol/L
Detergents	< 2 %

### Reagent (R2)

Standard (Phosphorus)	value on label
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## REAGENT PREPARATION AND STABILITY

Liquid and ready to use reagents, stable until the expiry date shown, if stored as indicated on the label and avoid contamination, evaporation and prolonged exposure to direct light.

Do not freeze the reagents.

Discard the reagent if signs of deterioration appear, such as the presence of particles and turbidity or failure to recover the values of certified control sera or Blank Absorbance (Abs) at 340 nm > 0.15 in 1 cm cuvette.

After opening the bottles, it is advisable to withdraw the necessary volume, to immediately close the bottles and store them in the fridge in order to avoid contamination, degradation from direct light and evaporation.

## SPECIMEN

Serum, heparinized plasma or 24 hours urine.

Do not use EDTA plasma. Avoid hemolysate or lipemic samples.

Separate the serum from the clot quickly.

Collect urine adding 10 mL of HCl at pH 3-4, then dilute the sample 1:10 (1+9) with distilled water.

### Stability:

In serum /plasma: 7 days at 4° - 25°C ; 3 months at -20°C

In urine: 2 days at 4° - 25°C at pH <5

Discard contaminated specimens.

## PROCEDURE

Wavelength: 340 nm (334-365)

Temperature: 37° C

Measurement: against distilled water

### Pipette as follow:

R1 Reagent 1000 µL

Sample, Std / Cal /H<sub>2</sub>O 10 µL

Mix and incubate for 5 minutes. Read Absorbance within 60 minutes.

## CALCULATION

### Serum / Plasma:

$$\text{Phosphorus} = \frac{\text{Abs Sample} - \text{Abs Blank Reagent}}{\text{Abs Std/Cal} - \text{Abs Blank Reagent}} \times \text{Conc. Std/Cal}$$

### Urine:

Calculate as for the serum/plasma and multiply the result by 10 (initial sample dilution).

Phosphorus [mg/24h] = Urinary Phosphorus x Urine Volume 24 h (dL)

Phosphorus [mmol/24h] = Urinary Phosphorus x Urine Volume 24 h (L)

Conversion factor: Phosphorus [mg/dL] x 0.3229 = Phosphorus [mmol/L]

## CALIBRATION

The results depend on the accuracy of the calibration, on the correct setting of the test on the instrument, on the correct volumetric ratio of reagent / sample and on the correct analysis temperature.

As an alternative to the standard included in the package, it is possible to use **MTD Calibrator:**

**Chemistry Multicalibrator: REF CAL1010 (10 x 3 mL)**

## QUALITY CONTROL

Normal and abnormal control sera of known concentration should be analysed routinely with each group of unknown samples utilizing **MTD Diagnostics** Quality Control Material:

**Chemistry Control N - REF CNN1010** 10 x 5 mL (Level 1)

**Chemistry Control P - REF CNP1020** 10 x 5 mL (Level 2)

## EXPECTED VALUES

### Serum / Plasma:

	[mg/dL]	[mmol/L]
Adults	2.6 - 4.5	0.84 - 1.45
Children / Adolescents:		
1 - 30 days	3.9 - 7.7	1.25 - 2.50
1 - 12 months	3.5 - 6.6	1.15 - 2.15
1 - 12 years	3.2 - 5.7	1.05 - 1.85
13 - 18 years	2.8 - 5.0	0.90 - 1.61

Urine: 400 - 1300 mg/24 h (123.16 - 419.17 mmol/24 h)

Each laboratory should establish a range of expected values based on its patient population and, if necessary, determine its own reference interval. For diagnostic purposes, results should always be assessed together with the patient's medical history, clinical examination and other results.

## PERFORMANCE

### PRECISION:

Low Level: Samples= 20; Average = 2.02; S.D. = 0.03; CV = 1.61%

High Level: Samples = 20; Average = 5.82; S.D. = 0.05; CV = 0.86%

**ACCURACY (CORRELATION):** A comparison between this method (x) and a certified method of trade (y) gave the following correlation:

$$y=1.016x - 0.150 ; r=1.000$$

**SENSITIVITY:** 0.2 mg/dL

**LINEARITY:** 0.2 – 18.0 mg/dL

## SPECIFICITY / INTERFERENCES

No interference was observed by ascorbic acid up to 18 mg/dL, bilirubin up to 20 mg/dL, haemoglobin up to 0,15 mg/dL and lipemia up to 1000 mg/dL triglycerides.

## NOTES

1. 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 acid and water before using.
2. This method may be used with different instruments. Any application to an instrument should be validated to demonstrate that results meet the performance characteristics of the method. It is recommended to validate periodically the instrument. Contact your distributor for any question on the application method.
3. Clinical diagnosis should not be made on findings of a single test result, but should integrate both clinical and laboratory data.

## PRECAUTIONS

R1 contains SULPHURIC ACID (H<sub>2</sub>SO<sub>4</sub>) – 210 mmol/L - CAS 7664-93-9 - C R35 (H314) - Xi R36/37/38 (H319 – H335 – H315) R35 (H314): Causes severe burns

R36/37/38 (H319 – H335 – H315): Irritating to eyes, respiratory system and skin.

The product contains dangerous substances or mixtures according to the EC regulation n ° 1272/2008 (CLP), therefore it needs the special labeling required by the aforementioned regulation. The product is labeled according to the directive for CE marking (98/79 / CE).

Sodium Azide less than 0.1%.

However, in compliance with the normal prudential rules that everyone has to keep managing any chemical or laboratory reagent, in case of contact of reagents with the operator, you must apply the following first aid:

S26 (P305 – P351 – P338): In case of contact with eyes, rinse immediately with plenty of water and seek medical advice.

S28 (P302 – P352): After contact with skin, wash immediately with plenty of water.

S36/37/39 (P280): Wear suitable protective clothing, gloves and eye/face protection.

S46 (P301 – P310): If swallowed, seek medical advice immediately and show container or label. If victim is conscious and alert, wash out mouth with water.

S56 (P273): Dispose of this material and its container at hazardous or special waste collection point.

S63 (P304 – P340): In case of accident by inhalation: remove casualty to fresh air and keep at rest. If breathing is difficult, give oxygen and get medical aid.

All samples, calibrators and controls should be treated as potentially infectious material capable of transmitting HIV and hepatitis.

FOR MORE INFORMATION, REQUEST SAFETY DATA SHEET FOR REAGENT (MSDS) AT THE MANUFACTURER.

## SIMBOLOGY

CE	CE Mark (EC Directive 98/79)		
	In Vitro Diagnostic		Temperature Limitation
	Consult instructions for use		Contains sufficient for <n> test
	Catalog Number		Use By
	Batch Code		Manufacturer

## BIBLIOGRAPHY

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