

N BIO - PHOSPHORUS

(Phosphomolybdate UV method)

KIT NAME	KIT SIZE	CAT. NO
N BIO - Phosphorus	2 X 50 ml	MPHO02050M



INTRODUCTION

Phosphorus is present in all body cells as a component of nucleic acids, phospholipids and phosphoproteins. Phosphorus is essential for intracellular storage and conversion of energy (ATP, creatine phosphate) and participates in carbohydrates metabolism. In the blood phosphorus is present as a mixture of inorganic phosphates H₂PO₄⁻² and H₂PO₄⁻. Besides phosphorus and calcium constitute mineral portion of bone. Continuous flux of phosphorus in organism is controlled by parathyroid hormone (PTH), vitamin D and calcitonin. Phosphorus serum level abnormalities are caused usually by disorders of vitamin D metabolism or parathyroid and kidney diseases.

METHOD PRINCIPLE

Direct phosphomolybdate reaction without deproteinization. Phosphate ions form with molybdate ions in acid solution proportional amounts of unreduced phosphomolybdate complex. The concentration of the complex formed is determined by measuring its absorbance $\lambda=340$ nm.

KIT CONTENTS

Reagent Name	MPHO02050M
R1 - Phosphorus Reagent	2 x 50 ml
R2 - Standard	1 vial

Refer standard value mentioned in the vial.

WORKING REAGENT PREPARATION AND STABILITY

The reagent is ready to use.

The reagent is stable up to the kit expiry date printed on the package when stored at 2-8°C. The reagents are stable for 8 weeks on board the analyser at 2-10°C. Protect from light, avoid contamination.

CONCENTRATIONS IN THE TEST

Ammonium molybdate	0.4 mmol/l
Sulphuric acid	100 mmol/l
Hydrochloric acid	100 mmol/l

WARNINGS AND NOTES

- Product for in vitro diagnostic use only.
- Contaminated glassware is the greatest source of error. Disposable plastic ware is recommended for the test.
- The reagent is usable when its absorbance is less than 0.350 (read against distilled water, wavelength $\lambda=340$ nm, cuvette l=1 cm, at temp. 25°C).
- Reagent 1-PHOSPHORUS is classified as an irritant, Reagent 1-MG is classified as an irritant.

Ingredients: sulphuric acid

Xi - Irritant

R 36/38: Irritating to eyes and skin

S 26-28-30-45: In case of contact with eyes, rinse

immediately with plenty of water and seek medical advice. After contact with skin, wash immediately with plenty of water. Never add water to this product. In case of accident or if you feel unwell, seek medical advice immediately (show the label where possible).



ADDITIONAL EQUIPMENT

- Automatic analyzer or photometer able to read at 340 nm (Hg 365 nm, 334 nm)
- Thermostat at 37°C
- General laboratory equipment.

SPECIMEN

Serum, heparinized plasma (recommended: heparine lithium, sodium or ammonium salt) free from hemolysis, 24-hours urine. Serum is the preferred specimen, Level of inorganic phosphate in heparinized plasma is about 0.2 to 0.3 mg/dl lower than in serum.

Serum should be separated from red blood cells as soon as possible after blood collection, because erythrocytes contain several times higher phosphate concentration than normal serum. Urine preparation: to prevent phosphate precipitation in urine, specimens should be collected in HCL, 20-30 ml of 6 mol/l for 24-h specimen. Then dilute 1 part of acidified urine with 10 parts of distilled water. Multiply the result by the dilution factor.

Serum and plasma can be stored up to 7 days at 2-8°C. For longer storage samples should be frozen at -20°C.

24-hours urine samples can be stored up to 7 days at 2-8°C. Nevertheless it is recommended to perform the assay with freshly collected samples.

PROCEDURE

These reagents may be used both for manual assay and in several automatic analysers. Applications for them are available on

Wavelength	340 nm (Hg 365 nm, 334 nm)
Temperature	20-25°C / 37°C
Cuvette	1 cm

Pipette into the cuvette:

Reagent	Blank (B)	Standard (S)	Test (T)
R1 Phosphorous Reagent	1000 μ l	1000 μ l	1000 μ l
Bring upto the temperature of determination, Then add			
Distilled Water	10 μ l		
R2 - Standard		10 μ l	
Sample			10 μ l

Mix well, incubate for 5 min. at the determination temperature. Read the absorbance of test A(T) and standard A(S) against blank(B). The absorbance is stable within 60 minutes.

CALCULATION

Phosphorus concentration = A(T) / A(S) x standard concentration

REFERENCE VALUES

Age 0 to 10 days	4.5 - 9.0 mg/dl
10 days to 24 Months	4.5 - 6.7 mg/dl
24 Months to 2 Years	4.5 - 5.5 mg/dl
12 to 20 Years	2.7 - 4.5 mg/dl
Above 60 years Male	2.3 - 3.7 mg/dl
Above 60 Years Female	2.8 - 4.1 mg/dl
Urine	0.4 - 1.3 mg / 24 hours

It is recommended for each laboratory to establish its own reference ranges for local population.

QUALITY CONTROL

To Ensure adequate quality control, each run should include assayed normal and abnormal controls. If commercial controls are not available it is recommended that known value samples be aliquoted, frozen and used as controls.

For Fully Automated analyzers by using multi-alibrator or phosphorus standard, the calibration curve can plot and the same should be prepared every 6 weeks or with change of reagent lot number.

PERFORMANCE CHARACTERISTICS

-Sensitivity / Limit of Quantitation: 0.25 mg/dl

-Linearity: up to 15 mg/dl (4.85 mmol/l). For higher concentration of phosphorus dilute the sample with 0.9% NaCl and repeat the assay. Multiply the result by dilution factor.

-Specificity / Interferences

Haemoglobin up to 2.5 g/dl, ascorbate up to 62 mg/l, bilirubin up to 20 mg/dl and triglycerides up to 500 mg/dl do not interfere with the test.

WASTE MANAGEMENT

Please refer to local legal requirements.

LITERATURE

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SYSTEM PARAMETERS

Method	End Point
Wavelength	340 nm
Zero Setting	Reagent Blank
Temperature Setting	25° C / 37° C
Incubation Temperature	37° C
Incubation Time	5 mins
Delay Time	----
Read Time	----
No. of Reading	----
Interval Time	----
Sample Volume	0.01 ml (10 ul)
Reagent Volume	1.0 ml (1000 ul)
Standard Concentration	Refer Standard vial
Units	mg/dl
Factor	----
Reaction Slope	Increasing
Linearity	15 mg/dl



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