MR.PHOSPHORUS
DIAGNOSTIC KIT
FOR DETERMINATION OF
INORGANIC PHOSPHORUS
CONCENTRATION

Kit name          Kit size       Cat. No
------------------ ----------------- ---------------------
Mr.Phosphorus mini 2 x 25 ml   GB22MR
Mr.Phosphorus 100   2 x 50 ml    GB23MR

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 at 340 nm.

REAGENTS
Package
Mr.Phosphorus mini Mr.Phosphorus 100
R1-Phosphorus       2 x 25 ml   2 x 50 ml
R2-STANDARD        1 vial      1 vial

R2-STANDARD is phosphorus ions standard solution: 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 λ=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: heparin 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 (0.06 – 0.10 mmol/l) 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.

Urinary 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 request.

Manual procedure

wavelength
340 nm (Hg 365 nm, 334 nm)
temperature
20-25°C / 37°C
cuvette
1 cm

Pipette into the cuvette:

<table>
<thead>
<tr>
<th></th>
<th>blank (B)</th>
<th>test (T)</th>
<th>standard (S)</th>
</tr>
</thead>
<tbody>
<tr>
<td>R1-Phosphorus</td>
<td>1000 μl</td>
<td>1000 μl</td>
<td>1000 μl</td>
</tr>
</tbody>
</table>

Bring up to the temperature of determination. Then add:

<table>
<thead>
<tr>
<th></th>
<th>standard</th>
<th>sample</th>
<th>distilled water</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th></th>
<th>serum / plasma</th>
<th>mg/dl</th>
<th>mmol/l</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age 0 - 10 days</td>
<td>4.5 – 9.0</td>
<td>1.45 – 2.91</td>
<td></td>
</tr>
<tr>
<td>10 d – 24 months</td>
<td>4.5 – 6.7</td>
<td>1.45 – 2.16</td>
<td></td>
</tr>
<tr>
<td>24 mon - 2 years</td>
<td>4.5 – 5.5</td>
<td>1.45 – 1.78</td>
<td></td>
</tr>
<tr>
<td>12 -20 years</td>
<td>2.7 – 4.5</td>
<td>0.87 – 1.45</td>
<td></td>
</tr>
<tr>
<td>&gt; 60 years male</td>
<td>2.3 – 3.7</td>
<td>0.74 – 1.20</td>
<td></td>
</tr>
<tr>
<td>&gt; 60 years female</td>
<td>2.8 – 4.1</td>
<td>0.90 – 1.32</td>
<td></td>
</tr>
<tr>
<td>24-hours urine:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>mg/24h</td>
<td>mmol/24h</td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.4 – 1.3</td>
<td>12.9 – 42.0</td>
<td></td>
</tr>
</tbody>
</table>

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

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Mr.Phosphorus page 1
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 multicalibrators or phosphorus standard, the calibration curve can plot and the same should be prepared every 6 weeks or with change of reagent lot number.

Sensitivity / Limit of Quantitation: 0.25 mg/dl (0.08 mmol/l).

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

SYSTEM PARAMETERS
<table>
<thead>
<tr>
<th>Method</th>
<th>Endpoint</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wavelength</td>
<td>340 nm</td>
</tr>
<tr>
<td>Zero Setting</td>
<td>Reagent Blank</td>
</tr>
<tr>
<td>Temperature Setting</td>
<td>25°C/ 37°C</td>
</tr>
<tr>
<td>Incubation Temperature</td>
<td>37°C</td>
</tr>
<tr>
<td>Incubation Time</td>
<td>5 mins</td>
</tr>
<tr>
<td>Delay time</td>
<td>----</td>
</tr>
<tr>
<td>Read time</td>
<td>----</td>
</tr>
<tr>
<td>No. of Reading</td>
<td>----</td>
</tr>
<tr>
<td>Interval time</td>
<td>----</td>
</tr>
<tr>
<td>Sample Volume</td>
<td>0.01 ml (10 ul)</td>
</tr>
<tr>
<td>Reagent Volume</td>
<td>1.0 ml (1000 ul)</td>
</tr>
<tr>
<td>Standard Concentration</td>
<td>Refer Standard vial</td>
</tr>
<tr>
<td>Units</td>
<td>mg/dl</td>
</tr>
<tr>
<td>Factor</td>
<td>----</td>
</tr>
<tr>
<td>Reaction slope</td>
<td>Increasing</td>
</tr>
<tr>
<td>Linearity</td>
<td>15 mg/dl</td>
</tr>
</tbody>
</table>

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