

# PHOS2

Phosphate (Inorganic) ver.2

## Order information

REF	CONTENT	Analizers on which <b>cobas c</b> pack can be used
03183793 122	Phosphate (Inorganic) ver.2 (250 tests)	System-ID 07 6614 3 COBAS INTEGRA 400 plus COBAS INTEGRA 800
10759350 190	Calibrator f.a.s. (12 x 3 mL)	System-ID 07 3718 6
10759350 360	Calibrator f.a.s. (12 x 3 mL, for USA)	System-ID 07 3718 6
10171743 122	Precinorm U (20 x 5 mL)	System-ID 07 7997 0
10171778 122	Precipath U (20 x 5 mL)	System-ID 07 7998 9
12149435 122	Precinorm U plus (10 x 3 mL)	System-ID 07 7999 7
12149435 160	Precinorm U plus (10 x 3 mL, for USA)	System-ID 07 7999 7
12149443 122	Precipath U plus (10 x 3 mL)	System-ID 07 8000 6
12149443 160	Precipath U plus (10 x 3 mL, for USA)	System-ID 07 8000 6
05117003 190	PreciControl ClinChem Multi 1 (20 x 5 mL)	System-ID 07 7469 3
05947626 190	PreciControl ClinChem Multi 1 (4 x 5 mL)	System-ID 07 7469 3
05947626 160	PreciControl ClinChem Multi 1 (4 x 5 mL, for USA)	System-ID 07 7469 3
05117216 190	PreciControl ClinChem Multi 2 (20 x 5 mL)	System-ID 07 7470 7
05947774 190	PreciControl ClinChem Multi 2 (4 x 5 mL)	System-ID 07 7470 7
05947774 160	PreciControl ClinChem Multi 2 (4 x 5 mL, for USA)	System-ID 07 7470 7

## English

### System information

Test PHOS2, test ID 0-614 (serum, plasma)

Test PHOU2, test ID 0-514 (urine)

### Intended use

In vitro test for the quantitative determination of the inorganic phosphate concentration in human serum, plasma, and urine on COBAS INTEGRA systems.

### Summary<sup>1,2,3,4,5</sup>

88 % of the phosphorus contained in the body is localized in bone in the form of calcium phosphate as the apatite  $\text{Ca}^{2+}[\text{Ca}_3(\text{PO}_4)_2]_3^{2-}$ . The remainder is involved in intermediary carbohydrate metabolism and in physiologically important substances such as phospholipids, nucleic acids and ATP. Phosphorus occurs in blood in the form of inorganic phosphate and in organically bound phosphoric acid. The small amount of extracellular organic phosphorus is found almost exclusively in the form of phospholipids.

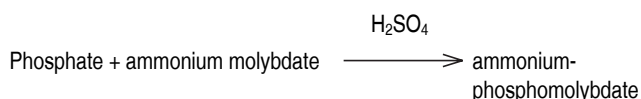
The ratio of phosphate to calcium in the blood is approximately 6:10. An increase in the level of phosphorus causes a decrease in the calcium level. The mechanism is influenced by interactions between parathormone and vitamin D. Hypoparathyroidism, vitamin D intoxication and renal failure with decreased glomerular phosphate filtration give rise to hyperphosphatemia. Hypophosphatemia occurs in rickets, hyperparathyroidism and Fanconi's syndrome.

The preferred method for the determination of inorganic phosphorus is based on the formation of ammonium phosphomolybdate with subsequent reduction to molybdenum blue. Reagent stability problems often occur with this method. The method presented here is based on the reaction of phosphate with ammonium molybdate to form ammonium phosphomolybdate without reduction. The addition of an accelerator gives rise to a more rapid rate of reaction and the application of sample blanking yields more precise results.

### Test principle<sup>5</sup>

Endpoint method with sample blanking

Inorganic phosphate forms an ammonium phosphomolybdate complex having the formula  $(\text{NH}_4)_3[\text{PO}_4(\text{MoO}_3)_{12}]$  with ammonium molybdate in the presence of sulfuric acid.



The concentration of phosphomolybdate formed is directly proportional to the inorganic phosphate concentration. It is determined by measuring the increase in absorbance at 340 nm.

### Reagents - working solutions

R1 Sulfuric acid 0.36 mol/L, detergent

SR Ammonium molybdate 3.5 mmol/L, Sulfuric acid 0.36 mol/L, Sodium chloride 150 mmol/L

R1 is in position B and SR is in position C

### Precautions and warnings

Pay attention to all precautions and warnings listed in Section 1 / Introduction of this Method Manual.

For USA: For prescription use only.

This kit contains components classified as follows in accordance with the Regulation (EC) No. 1272/2008:



Warning

H290 May be corrosive to metals.

### Prevention:

P234 Keep only in original container.

### Response:

P390 Absorb spillage to prevent material damage.

Product safety labeling primarily follows EU GHS guidance.

Contact phone: all countries: +49-621-7590, USA: 1-800-428-2336

### Reagent handling

Ready for use

### Storage and stability

Shelf life at 2-8 °C

See expiration date on  
**cobas c** pack label

COBAS INTEGRA 400 plus systems

On-board in use at 10-15 °C

12 weeks

COBAS INTEGRA 800 systems

On-board in use at 8 °C

12 weeks

# PHOS2

Phosphate (Inorganic) ver.2

**cobas**<sup>®</sup>  
Substrates

## Specimen collection and preparation

For specimen collection and preparation only use suitable tubes or collection containers.

Only the specimens listed below were tested and found acceptable.

Serum

Plasma: Heparin (Li-, Na-, NH<sub>4</sub><sup>+</sup>-) or EDTA (K<sub>2</sub>-, K<sub>3</sub>-) plasma

The sample types listed were tested with a selection of sample collection tubes that were commercially available at the time of testing, i.e. not all available tubes of all manufacturers were tested. Sample collection systems from various manufacturers may contain differing materials which could affect the test results in some cases. When processing samples in primary tubes (sample collection systems), follow the instructions of the tube manufacturer.

## Urine

Urine should be collected in an acid-washed, detergent-free container. Acidify with hydrochloric acid after collection (pH < 3).<sup>6,7</sup>

Urine samples are automatically prediluted 1:11 (1+10) with water by the instrument.

Stability in *serum/plasma*:<sup>8</sup> 24 hours at 15-25 °C  
4 days at 2-8 °C  
1 year at (-15)-(-25) °C

Stability in *urine*:<sup>6,7</sup> 6 months at 2-8 °C (when acidified)

24-hour urine: Store cooled during collection.

Centrifuge samples containing precipitates before performing the assay.

## Materials provided

See "Reagents – working solutions" section for reagents.

## Assay

For optimum performance of the assay follow the directions given in this document for the analyzer concerned. Refer to the appropriate operator's manual for analyzer-specific assay instructions.

## Application for serum, plasma, and urine

### COBAS INTEGRA 400 plus test definition

Measuring mode	Absorbance
Abs. calculation mode	Endpoint
Reaction direction	Increase
Wavelength A/B	340/659 nm
Calc. first/last	33/63
Unit	mmol/L

### Serum, plasma

Reaction mode R1-S-SR

### Urine

Reaction mode D-R1-S-SR

Predilution factor 11

### Pipetting parameters

<i>Serum, plasma, and urine</i>	Diluent (H <sub>2</sub> O)	
R1	90 µL	
Sample	2.5 µL	27.5 µL
SR	38 µL	
Total volume	158 µL	

### COBAS INTEGRA 800 test definition

Measuring mode	Absorbance
Abs. calculation mode	Endpoint

Reaction direction	Increase
Wavelength A/B	340/659 nm
Calc. first/last	44/98
Unit	mmol/L

### Serum, plasma

Reaction mode R1-S-SR

### Urine

Reaction mode D-R1-S-SR

Predilution factor 11

### Pipetting parameters

<i>Serum, plasma, and urine</i>	Diluent (H <sub>2</sub> O)	
R1	90 µL	
Sample	2.5 µL	27.5 µL
SR	38 µL	
Total volume	158 µL	

## Calibration

### *Serum, plasma, and urine*

Calibrator	Calibrator f.a.s. Use deionized water as zero calibrator.
Calibration mode	Linear regression
Calibration replicate	Duplicate recommended
Calibration interval	Each lot and as required following quality control procedures

Traceability: This method has been standardized against NERL primary reference material.

For USA: This method has been standardized against NIST traceable primary reference material.

## Quality control

Quality control serum, plasma	Precinorm U, Precinorm U plus Precipath U or Precipath U plus
Quality control urine	Quantitative urine controls are recommended for routine quality control.
Control interval	24 hours recommended
Control sequence	User defined
Control after calibration	Recommended

For quality control, use control materials as listed in the "Order information" section. In addition, other suitable control material can be used.

The control intervals and limits should be adapted to each laboratory's individual requirements. Values obtained should fall within the defined limits. Each laboratory should establish corrective measures to be taken if values fall outside the defined limits.

Follow the applicable government regulations and local guidelines for quality control.

## Calculation

COBAS INTEGRA analyzers automatically calculate the analyte concentration of each sample. For more details, please refer to Data Analysis in the Online Help (COBAS INTEGRA 400 plus/800 analyzers).

Conversion factor: mmol/L × 3.10 = mg/dL

## Limitations - interference

Criterion: Recovery within ± 10 % of initial value.

*Serum, plasma*

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Icterus:<sup>9</sup> No significant interference up to an I index of 51 (approximate conjugated bilirubin concentration: 872 µmol/L or 51 mg/dL). No significant interference with unconjugated bilirubin.

Hemolysis:<sup>9</sup> No significant interference up to an H index of 420 (approximate hemoglobin concentration: 261 µmol/L or 420 mg/dL).

Lipemia (Intralipid):<sup>9</sup> No significant interference up to an Intralipid level of 1000 mg/dL. There is poor correlation between turbidity and triglycerides concentration.

Drugs: No interference was found at therapeutic concentrations using common drug panels.<sup>10,11</sup> **Exception:** Phospholipids contained in liposomal drug formulations (e.g. AmBisome) may be hydrolyzed in the test due to the acidic reaction pH and thus lead to elevated phosphate results.<sup>12</sup>

In very rare cases, gammopathy, in particular type IgM (Waldenström's macroglobulinemia), may cause unreliable results.<sup>13</sup>

## Urine

Drugs: No interference was found at therapeutic concentrations using common drug panels.<sup>11</sup>

For diagnostic purposes, the results should always be assessed in conjunction with the patient's medical history, clinical examination and other findings.

## ACTION REQUIRED

**Special Wash Programming:** The use of special wash steps is mandatory when certain test combinations are run together on COBAS INTEGRA analyzers. Refer to the CLEAN Method Sheet for further instructions and for the latest version of the Extra wash cycle list.

**Where required, special wash/carry-over evasion programming must be implemented prior to reporting results with this test.**

## Limits and ranges

### Measuring range

#### Serum/plasma

0.1-6.46 mmol/L (0.31-20 mg/dL)

Determine samples having higher concentrations via the rerun function. Dilution of samples via the rerun function is a 1:10 dilution. Results from samples diluted using the rerun function are automatically multiplied by a factor of 10.

#### Urine

1.1-92 mmol/L (3.41-285 mg/dL)

Determine samples having higher concentrations via the rerun function. Dilution of samples via the rerun function is a 1:5 dilution. Results from samples diluted using the rerun function are automatically multiplied by a factor of 5.

## Lower limits of measurement

#### Serum/plasma

Lower detection limit of the test:

0.1 mmol/L (0.31 mg/dL)

The lower detection limit represents the lowest measurable analyte level that can be distinguished from zero. It is calculated as the value lying 3 standard deviations above that of the lowest standard (standard 1 + 3 SD, repeatability, n = 21).

#### Urine

Lower detection limit of the test:

1.1 mmol/L (3.41 mg/dL)

The lower detection limit represents the lowest measurable analyte level that can be distinguished from zero. It is calculated as the value lying 3 standard deviations above that of the lowest standard (standard 1 + 3 SD, repeatability, n = 21).

## Expected values

#### Serum, plasma

##### Adults<sup>14</sup>

0.81-1.45 mmol/L (2.5-4.5 mg/dL)

##### Children<sup>15</sup>

Age	Male mmol/L (mg/dL)	Female mmol/L (mg/dL)
1-30 days	1.25-2.25 (3.9-6.9)	1.40-2.50 (4.3-7.7)

1-12 months	1.15-2.15 (3.5-6.6)	1.20-2.10 (3.7-6.5)
1-3 years	1.00-1.95 (3.1-6.0)	1.10-1.95 (3.4-6.0)
4-6 years	1.05-1.80 (3.3-5.6)	1.05-1.80 (3.2-5.5)
7-9 years	0.95-1.75 (3.0-5.4)	1.00-1.80 (3.1-5.5)
10-12 years	1.05-1.85 (3.2-5.7)	1.05-1.70 (3.3-5.3)
13-15 years	0.95-1.65 (2.9-5.1)	0.90-1.55 (2.8-4.8)
16-18 years	0.85-1.60 (2.7-4.9)	0.80-1.55 (2.5-4.8)

Roche has not evaluated reference ranges in a pediatric population.

## Urine

1st morning urine<sup>16</sup> 13-44 mmol/L (40-136 mg/dL)

24h urine<sup>6</sup> 13-42 mmol/d (0.4-1.3 g/d)

Each laboratory should investigate the transferability of the expected values to its own patient population and if necessary determine its own reference ranges.

## Specific performance data

Representative performance data on the analyzers are given below. Results obtained in individual laboratories may differ.

## Precision

Precision was determined using human samples and controls in an internal protocol with repeatability (n = 21) and intermediate precision (1 aliquot per run, 1 run per day, 21 days). The following results were obtained:

### Serum and plasma

	Level 1	Level 2
Mean	1.17 mmol/L (3.63 mg/dL)	2.01 mmol/L (6.23 mg/dL)
CV repeatability	1.3 %	1.4 %
Mean	1.17 mmol/L (3.63 mg/dL)	2.00 mmol/L (6.20 mg/dL)
CV intermediate precision	2.5 %	2.4 %

Precision was determined using human samples and controls in an internal protocol with repeatability (n = 21) and intermediate precision (1 aliquot per run, 1 run per day, 10 days). The following results were obtained:

## Urine

	Level 1	Level 2
Mean	13.9 mmol/L (43.1 mg/dL)	27.6 mmol/L (85.6 mg/dL)
CV repeatability	1.0 %	0.7 %
Mean	13.9 mmol/L (43.1 mg/dL)	27.7 mmol/L (85.9 mg/dL)
CV intermediate precision	1.7 %	1.1 %

## Method comparison

Inorganic phosphate values obtained on a COBAS INTEGRA 700 analyzer with the COBAS INTEGRA Phosphate (Inorganic) ver.2 reagent (y) were compared to those determined with the same reagent on a Roche/Hitachi 917 analyzer (x) and to the previous reagent (PHOS) on a COBAS INTEGRA 700 analyzer (x).

### Serum and plasma

<b>Roche/Hitachi 917 analyzer</b>	Sample size (n) = 100
Passing/Bablok <sup>17</sup>	Linear regression
y = 1.043x + 0.022 mmol/L	y = 1.040x + 0.025 mmol/L
r = 0.955	r = 1.000
SD (md 95) = 0.040	Sy.x = 0.018

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The sample concentrations were between 0.572 to 5.69 mmol/L (1.77 to 17.7 mg/dL).

<b>COBAS INTEGRA 700 analyzer</b>	Sample size (n) = 96
Passing/Bablok <sup>17</sup>	Linear regression
$y = 1.029x - 0.047$ mmol/L	$y = 1.040x - 0.067$ mmol/L
$r = 0.942$	$r = 0.999$
SD (md 95) = 0.077	Sy.x = 0.032

The sample concentrations were between 0.619 to 4.76 mmol/L (1.92 to 14.9 mg/dL).

## Urine

<b>Roche/Hitachi 917 analyzer</b>	Sample size (n) = 86
Passing/Bablok <sup>17</sup>	Linear regression
$y = 1.052x - 0.0235$ mmol/L	$y = 1.044x - 0.028$ mmol/L
$r = 0.983$	$r = 1.000$
SD (md 95) = 0.743	Sy.x = 0.349

The sample concentrations were between 6.08 to 89.4 mmol/L (18.9 to 277 mg/dL).

<b>COBAS INTEGRA 700 analyzer</b>	Sample size (n) = 96
Passing/Bablok <sup>17</sup>	Linear regression
$y = 1.000x - 0.399$ mmol/L	$y = 1.002x - 0.405$ mmol/L
$r = 0.989$	$r = 1.000$
SD (md 95) = 0.396	Sy.x = 0.180

The sample concentrations were between 6.08 to 44.8 mmol/L (18.9 to 139 mg/dL).

## References

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A point (period/stop) is always used in this Method Sheet as the decimal separator to mark the border between the integral and the fractional parts of a decimal numeral. Separators for thousands are not used.

## Symbols

Roche Diagnostics uses the following symbols and signs in addition to those listed in the ISO 15223-1 standard:

CONTENT

Contents of kit



Volume after reconstitution or mixing

GTIN

Global Trade Item Number

## FOR US CUSTOMERS ONLY: LIMITED WARRANTY

Roche Diagnostics warrants that this product will meet the specifications stated in the labeling when used in accordance with such labeling and will be free from defects in material and workmanship until the expiration date printed on the label. THIS LIMITED WARRANTY IS IN LIEU OF ANY OTHER WARRANTY, EXPRESS OR IMPLIED, INCLUDING ANY IMPLIED WARRANTY OF MERCHANTABILITY OR FITNESS FOR PARTICULAR PURPOSE. IN NO EVENT SHALL ROCHE DIAGNOSTICS BE LIABLE FOR INCIDENTAL, INDIRECT, SPECIAL OR CONSEQUENTIAL DAMAGES.

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