

**Phosphate (Inorganic) ver.2****Order information**

REF	CONTENT	Analyzer(s) on which <b>cobas c</b> pack(s) can be used
03183793 122	Phosphate (Inorganic) ver.2 250 tests	System-ID 07 6614 3 Roche/Hitachi <b>cobas c</b> 311, <b>cobas c</b> 501/502
10759350 190	Calibrator f.a.s. (12 x 3 mL)	Code 401
10759350 360	Calibrator f.a.s. (12 x 3 mL, for USA)	Code 401
12149435 122	Precinorm U plus (10 x 3 mL)	Code 300
12149435 160	Precinorm U plus (10 x 3 mL, for USA)	Code 300
12149443 122	Precipath U plus (10 x 3 mL)	Code 301
12149443 160	Precipath U plus (10 x 3 mL, for USA)	Code 301
10171743 122	Precinorm U (20 x 5 mL)	Code 300
10171735 122	Precinorm U (4 x 5 mL)	Code 300
10171778 122	Precipath U (20 x 5 mL)	Code 301
10171760 122	Precipath U (4 x 5 mL)	Code 301
05117003 190	PreciControl ClinChem Multi 1 (20 x 5 mL)	Code 391
05947626 190	PreciControl ClinChem Multi 1 (4 x 5 mL)	Code 391
05947626 160	PreciControl ClinChem Multi 1 (4 x 5 mL, for USA)	Code 391
05117216 190	PreciControl ClinChem Multi 2 (20 x 5 mL)	Code 392
05947774 190	PreciControl ClinChem Multi 2 (4 x 5 mL)	Code 392
05947774 160	PreciControl ClinChem Multi 2 (4 x 5 mL, for USA)	Code 392
04489357 190	Diluent NaCl 9 % (50 mL)	System-ID 07 6869 3

**English****System information**

For **cobas c** 311 analyzer:

**PHOS2:** ACN 714 (serum/plasma)

**SPHO2:** ACN 675 (STAT, reaction time: 7: serum/plasma)

**PHO2U:** ACN 716 (urine)

**SPH2U:** ACN 656 (STAT, reaction time: 7: urine)

For **cobas c** 501 analyzer:

**PHOS2:** ACN 714 (serum/plasma/urine)

**SPHO2:** ACN 675 (STAT, reaction time: 7: serum/plasma/urine)

For **cobas c** 502 analyzer:

**PHOS2:** ACN 8714 (serum/plasma)

**SPHO2:** ACN 8675 (STAT, reaction time: 7: serum/plasma)

**PHO2U:** ACN 8716 (urine)

**SPH2U:** ACN 8656 (STAT, reaction time: 7: urine)

**Intended use**

In vitro test for the quantitative determination of phosphorus in human serum, plasma and urine on Roche/Hitachi **cobas c** 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.

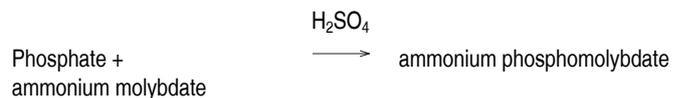
The preferred method for the determination of inorganic phosphorus is based on the formation of ammonium phosphomolybdate with subsequent reduction to molybdenum blue. Reagent stability problems often occur with this method. The method presented here is based on the reaction of phosphate with ammonium 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>

Molybdate UV.

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 and is measured photometrically.

**Reagents - working solutions**

**R1** Sulfuric acid: 0.36 mol/L; detergent

**R2** Ammonium molybdate: 3.5 mmol/L; sulfuric acid: 0.36 mol/L; sodium chloride: 150 mmol/L

R1 is in position B and R2 is in position C.

**Precautions and warnings**

For in vitro diagnostic use.

Exercise the normal precautions required for handling all laboratory reagents.

Disposal of all waste material should be in accordance with local guidelines. Safety data sheet available for professional user on request.

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:**

# PHOS2

## Phosphate (Inorganic) ver.2



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

#### PHOS2

Shelf life at 2-8 °C: See expiration date on **cobas c** pack label.

On-board in use and refrigerated on the analyzer: 12 weeks

#### Diluent NaCl 9 %

Shelf life at 2-8 °C: See expiration date on **cobas c** pack label.

On-board in use and refrigerated on the analyzer: 12 weeks

### 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: Li-heparin and K<sub>2</sub>-EDTA 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

Collect in detergent-free containers. Acidify with hydrochloric acid after collection (pH < 3).<sup>6,7</sup>

**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.

### Materials required (but not provided)

- See "Order information" section
- General laboratory equipment

### 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.

The performance of applications not validated by Roche is not warranted and must be defined by the user.

### Application for serum and plasma

#### cobas c 311 test definition

Assay type 2-Point End

Reaction time / Assay points 10 / 6-32 (STAT 7 / 6-32)

Wavelength (sub/main) 700/340 nm

Reaction direction Increase

Units mmol/L (mg/dL, mg/L)

Reagent pipetting Diluent (H<sub>2</sub>O)

R1 90 µL 28 µL

R2 38 µL –

#### Sample volumes

	Sample	Sample dilution	
		Sample	Diluent (NaCl)
Normal	2.5 µL	–	–
Decreased	12.5 µL	15 µL	135 µL
Increased	2.5 µL	–	–

#### cobas c 501 test definition

Assay type 2-Point End

Reaction time / Assay points 10 / 10-47 (STAT 7 / 10-47)

Wavelength (sub/main) 700/340 nm

Reaction direction Increase

Units mmol/L (mg/dL, mg/L)

Reagent pipetting Diluent (H<sub>2</sub>O)

R1 90 µL 28 µL

R2 38 µL –

#### Sample volumes

	Sample	Sample dilution	
		Sample	Diluent (NaCl)
Normal	2.5 µL	–	–
Decreased	12.5 µL	15 µL	135 µL
Increased	2.5 µL	–	–

#### cobas c 502 test definition

Assay type 2-Point End

Reaction time / Assay points 10 / 10-47 (STAT 7 / 10-47)

Wavelength (sub/main) 700/340 nm

Reaction direction Increase

Units mmol/L (mg/dL, mg/L)

Reagent pipetting Diluent (H<sub>2</sub>O)

R1 90 µL 28 µL

R2 38 µL –

#### Sample volumes

	Sample	Sample dilution	
		Sample	Diluent (NaCl)
Normal	2.5 µL	–	–
Decreased	12.5 µL	15 µL	135 µL
Increased	5 µL	–	–

### Application for urine

#### cobas c 311 test definition

Assay type 2-Point End

Reaction time / Assay points 10 / 6-32 (STAT 7 / 6-32)

Wavelength (sub/main) 700/340 nm

Reaction direction Increase

# PHOS2

## Phosphate (Inorganic) ver.2



Units	mmol/L (mg/dL, mg/L)	
Reagent pipetting	Diluent (H <sub>2</sub> O)	
R1	90 µL	28 µL
R2	38 µL	–

Sample volumes	Sample	Sample dilution	
		Sample	Diluent (NaCl)
Normal	2.5 µL	15 µL	150 µL
Decreased	2.5 µL	8 µL	168 µL
Increased	2.5 µL	15 µL	150 µL

### cobas c 501 test definition

Assay type	2-Point End	
Reaction time / Assay points	10 / 10-47 (STAT 7 / 10-47)	
Wavelength (sub/main)	700/340 nm	
Reaction direction	Increase	
Units	mmol/L (mg/dL, mg/L)	
Reagent pipetting	Diluent (H <sub>2</sub> O)	
R1	90 µL	28 µL
R2	38 µL	–

Sample volumes	Sample	Sample dilution	
		Sample	Diluent (NaCl)
Normal	2.5 µL	15 µL	150 µL
Decreased	2.5 µL	8 µL	168 µL
Increased	2.5 µL	15 µL	150 µL

### cobas c 502 test definition

Assay type	2-Point End	
Reaction time / Assay points	10 / 10-47 (STAT 7 / 10-47)	
Wavelength (sub/main)	700/340 nm	
Reaction direction	Increase	
Units	mmol/L (mg/dL, mg/L)	
Reagent pipetting	Diluent (H <sub>2</sub> O)	
R1	90 µL	28 µL
R2	38 µL	–

Sample volumes	Sample	Sample dilution	
		Sample	Diluent (NaCl)
Normal	2.5 µL	15 µL	150 µL
Decreased	2.5 µL	8 µL	168 µL
Increased	5 µL	15 µL	150 µL

### Calibration

Calibrators	S1: H <sub>2</sub> O S2: C.f.a.s.
Calibration mode	Linear
Calibration frequency	2-point calibration <ul style="list-style-type: none"> <li>• after reagent lot change</li> <li>• as required following quality control procedures</li> </ul>

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

#### Serum/plasma

For quality control, use control materials as listed in the "Order information" section.

In addition, other suitable control material can be used.

#### Urine

Quantitative urine controls are recommended for routine quality control.

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

Roche/Hitachi **cobas c** systems automatically calculate the analyte concentration of each sample.

Conversion factors:	mmol/L x 3.10 = mg/dL
	mmol/L x 31 = mg/L
	mg/L x 0.0323 = mmol/L

### Limitations - interference<sup>6</sup>

Criterion: Recovery within ± 10 % of initial value at a phosphate concentration of 0.87 mmol/L (2.7 mg/dL).

#### Serum/plasma

Icterus:<sup>9</sup> No significant interference up to an I index of 40 for conjugated and 60 for unconjugated bilirubin (approximate conjugated bilirubin concentration: 684 µmol/L or 40 mg/dL and approximate unconjugated bilirubin concentration: 1026 µmol/L or 60 mg/dL).

Hemolysis:<sup>9</sup> Significant positive interference at an H index > 300 (approximate hemoglobin concentration: 186 µmol/L or 300 mg/dL).

Note: This interference results from inorganic phosphates produced by the action of phosphatases on organic phosphates, both of which are released from the red cells upon hemolysis.

Lipemia (Intralipid):<sup>9</sup> No significant interference up to an L index of 1250. There is poor correlation between the L index (corresponds to 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 (eg 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 Roche/Hitachi **cobas c** systems. The latest version of the carry-over evasion list can be found with the NaOHD-SMS-SmpCln1+2-SCCS Method Sheets. For further instructions refer to the operator's manual. **cobas c** 502 analyzer: All special wash programming necessary for avoiding carry-over is available via the **cobas** link, manual input is not required.

**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**

0.10-6.46 mmol/L (0.31-20.0 mg/dL)

Determine samples having higher concentrations via the rerun function. Dilution of samples via the rerun function is a 1:2 dilution. Results from samples diluted using the rerun function are automatically multiplied by a factor of 2.

**Urine**

1.1-92 mmol/L (3.4-285 mg/dL)

Determine samples having higher concentrations via the rerun function. Dilution of samples via the rerun function is a 1:2 dilution. Results from samples diluted using the rerun function are automatically multiplied by a factor of 2.

**Lower limits of measurement****Lower detection limit of the test****Serum/plasma**

0.10 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**

1.1 mmol/L (3.4 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 d	1.25-2.25 (3.9-6.9)	1.40-2.50 (4.3-7.7)
1-12 m	1.15-2.15 (3.5-6.6)	1.20-2.10 (3.7-6.5)
1-3 y	1.00-1.95 (3.1-6.0)	1.10-1.95 (3.4-6.0)
4-6 y	1.05-1.80 (3.3-5.6)	1.05-1.80 (3.2-5.5)
7-9 y	0.95-1.75 (3.0-5.4)	1.00-1.80 (3.1-5.5)
10-12 y	1.05-1.85 (3.2-5.7)	1.05-1.70 (3.3-5.3)
13-15 y	0.95-1.65 (2.9-5.1)	0.90-1.55 (2.8-4.8)
16-18 y	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)24-hour 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.

**Serum/plasma:**

repeatability (n = 21), intermediate precision (3 aliquots per run, 1 run per day, 21 days);

**Urine:**

repeatability (n = 21), intermediate precision (3 aliquots per run, 1 run per day, 10 days).

The following results were obtained:

**Serum/plasma**

Repeatability	Mean mmol/L (mg/dL)	SD mmol/L (mg/dL)	CV %
Precinorm U	1.24 (3.84)	0.01 (0.03)	0.7
Precipath U	2.05 (6.36)	0.01 (0.03)	0.6
Human serum 1	2.68 (8.31)	0.02 (0.06)	0.6
Human serum 2	1.56 (4.84)	0.01 (0.03)	0.7

**Intermediate precision**

Mean mmol/L (mg/dL)	SD mmol/L (mg/dL)	CV %	
Precinorm U	1.23 (3.81)	0.02 (0.06)	1.4
Precipath U	2.04 (6.32)	0.02 (0.06)	1.2
Human serum 3	2.67 (8.28)	0.04 (0.12)	1.4
Human serum 4	1.55 (4.81)	0.02 (0.06)	1.4

**Urine**

Repeatability	Mean mmol/L (mg/dL)	SD mmol/L (mg/dL)	CV %
Control Level 1	10.2 (31.6)	0.1 (0.3)	1.4
Control Level 2	19.9 (61.7)	0.2 (0.6)	1.2
Human urine 1	40.9 (127)	0.4 (1)	1.0
Human urine 2	6.25 (19.4)	0.08 (0.2)	1.2

**Intermediate precision**

Mean mmol/L (mg/dL)	SD mmol/L (mg/dL)	CV %	
Control Level 1	10.0 (31.0)	0.2 (0.6)	1.6
Control Level 2	19.6 (60.8)	0.3 (0.9)	1.7
Human urine 3	40.4 (125)	0.5 (2)	1.3
Human urine 4	6.23 (19.3)	0.12 (0.4)	2.0

**Method comparison**

Inorganic phosphate values for human serum, plasma and urine samples obtained on a Roche/Hitachi **cobas c 501** analyzer (y) were compared with those determined using the corresponding reagent on a Roche/Hitachi 917 analyzer (x).

**Serum/plasma**

Sample size (n) = 150

Passing/Bablok<sup>17</sup>

y = 1.022x + 0.000 mmol/L

τ = 0.978

Linear regression

y = 1.023x - 0.002 mmol/L

r = 1.000

The sample concentrations were between 0.62 and 5.54 mmol/L (1.92 and 17.2 mg/dL).

**Urine**

Sample size (n) = 145

Passing/Bablok<sup>17</sup>

y = 0.976x - 0.053 mmol/L

τ = 0.967

Linear regression

y = 0.974x - 0.047 mmol/L

r = 0.999

The sample concentrations were between 1.61 and 91.5 mmol/L (4.99 and 284 mg/dL).

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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.

	Contents of kit
	Volume after reconstitution or mixing
	Global Trade Item Number

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