BIOLABO
www.biolabo.fr

MANUFACTURER:
BIOLABO S.A.S
Les Hautes Rives
02160, Maizy, France

Inorganic PHOSPHORUS U.V.Method

Reagent for quantitative determination of inorganic phosphate in human plasma and serum or urines

REF 80015 **R1** 2 x 125 mL **R2** 1 x 5 mL

CE

IVD

Made In France

I: corresponds to significant modifications

TECHNICAL SUPPORT AND ORDERS
Tel: (33) 03 23 25 15 50
support@biolabo.fr
Latest revision : www.biolabo.fr

INTENDED USE

This reagent is designated for professional use in laboratory (manual or automated method).
It allows the quantification of phosphorus in human serum and plasma, or urines to assess phosphorous homeostasis.

GENERALITIES (1) (2)

An adult human body contains approximately 600 g of phosphates expressed as phosphorus, of which about 85% is bound to calcium in bones and the rest principally in other tissue cells. Most phosphate present in cells is organic and incorporated into phospholipids, nucleic acids, and high energy compounds. Serum/plasma contains approximately 1% of total phosphate as inorganic phosphate, the fraction measured in routine biochemical analysis.

Elevation of phosphorus in serum/plasma is often associated with bone diseases, renal failures, hypoparathyroidism, hypervitaminosis D.

Decreased serum/plasma phosphorus concentrations are found in osteomalacia, vitamin D deficiency, primary hyperparathyroidism.

PRINCIPLE (4) (5)

Method without deproteinisation described by Daly and al. modified by Gamst O.K. and Try K.
In an acid medium, phosphate ions form a phosphomolybdic complex with the ammonium molybdate.The absorbance measured at 340 nm is proportional to the concentration of phosphate ions in the specimen.

I REAGENTS

R1 **Phosphorus** Molybdate Reagent

Ammonium Molybdate	0.63	mmol/L
Sulfuric acid	210	mmol/L
Surfactant		

EUH210: Safety Data Sheet (MSDS) available on request

R1 **Phosphorus** Standard

Phosphorus 5 mg/dL (1.61 mmol/L)

According to 1272/2008/EC regulation, these reagents are not classified as dangerous

I SAFETY CAUTIONS

- Refer to current Material Safety Data Sheet available on request or on www.biolabo.fr
- Verify the integrity of the contents before use.
- Waste disposal: Respect legislation in force in the country.
- All specimens or reagents of biological origin should be handled as potentially infectious. Respect legislation in force in the country.

Any serious incident that has occurred in connection with the device is notified to the manufacturer and the competent authority of the Member State in which the user and/or patient is based.

REAGENT PREPARATION

Ready for use

STABILITY AND STORAGE

Stored away from light, well caped in the original vial at 18-25°C, when used and stored as described, reagents are stable:

Unopened:

- Until expiry date stated on the label

Once opened

- Standard (vial R2): transfer requested quantity, well recap the vial and store at 18-25°C.
- Reagent is stable at least 6 months.
- Discard any reagent if cloudy or if absorbance at 340 nm > 0.800.
- Do not use after expiry date

SPECIMEN COLLECTION AND HANDLING (2)

Unhemolysed serum or plasma:
Separate from erythrocytes within 1 hour after collection.

- Phosphorus is stable in serum for:
  - ✓several days at 2-8°C.
  - ✓several months at –15°C.

24h Urines:

Collect in acid washed, detergent free container. Acidified urines (pH < 3 with concentrated hydrochloric acid) should be diluted (1+9) in demineralised water free from phosphorus before performing the assay.

- Phosphorus is stable in acidified urines for:
  - ✓6 months.

LIMITS (3)

To avoid contamination with environmental phosphorus, it is recommended to use disposal glass or plastic ware, carefully cleaned material with hydrochloric acid 1 N and well rinsed with distilled water.

For a more comprehensive review of factors affecting this assay refer to the publication of Young D.S.

I MATERIALS REQUIRED BUT NOT PROVIDED

1.Basic medical analysis laboratory equipment.
2.Spectrophotometer or Biochemistry Clinical Analyzer
3.Saline (Specimen Blank)

Manufacturer	Expiry date	In vitro diagnostic	Storage temperature	Dematerialized water	Biological risk
Product Reference	See Insert	Batch number	Store away from light	Sufficient for	Dilute with

CALIBRATION (6)

- REF 95015 BIOLABO Multicalibrator traceable *to SRM 3186*
- REF 80015 (vial R2) for urines and manual method only

The calibration frequency depends on proper instrument functions and on the preservation of the reagent.

REFERENCE INTERVALS (2)

In serum or plasma	(mg/dL)	(mmol/L)
In cord	3.7-8.1	[1.20-2.62]
Premature	5.4-10.9	[1.74-3.52]
0-10 days	4.5-9.0	[1.45-2.91]
10 days-24 months	4.5-6.7	[1.45-2.16]
24 months-12 years	4.5-5.5	[1.45-1.78]
12-60 years	2.7-4.5	[0.87-1.45]
> 60 years, male	2.3-3.7	[0.74-1.20]
> 60 years, female	2.8-4.1	[0.90-1.32]

In 24 h Urines	(mg/24 h)	(mmol/24 h)
Constant daily diet (*)	< 1000	[< 32.3]
Non-restricted diet	400-1300	[12.9-42.0]

Each laboratory should establish its own normal ranges for the population that it serves.

(*)Constant daily diet: 0.9 at 1.5 g P (29-48 mmol P) and 10 mg Ca/Kg (0.25 mmol Ca/Kg).

I PERFORMANCES

Serum: KENZA 240TX with specimen blank, at 37°C, 340 nm

Linearity Range: between 0.92 (LOQ) and 10 mg/dL

Above, dilute specimen with demineralised water free from phosphorus and re-assay taking into account dilution factor to calculate the result.

Precision:

Within-run N = 20	*Low level*	*Normal level*	*High level*
Mean (mg/dL)	1.86	3.89	7.15
S.D. mg/dL	0.02	0.06	0.08
C.V. %	0.9%	1.5%	1.2%

Between run N = 12	*Normal level*	*High level*
Mean (mg/dL)	3.88	8.05
S.D. mg/dL	0.05	0.14
C.V. %	1.3%	1.7%

Analytical Sensitivity: approx. 0.1079 abs for 1 mg/dL

Comparison study with commercially available method on human specimens between 1,5 and 10,8 mg/dL (n=74):

y = 0.990 x + 0.0504 r = 0.9948

Interferences:

Turbidity	No interference up to 0,240OD (7 mmol/L triglycerides)
Ascorbic acid	No interference up to 2500 mg/dL
Total bilirubin	No interference up to 450 µmol/L
Direct bilirubin	No interference up to 90 µmol/L
Haemoglobin	Positive interference from 170 µmol/L
Glucose	No interference up to 1000 mg/dL

Other substances may interfere (see § Limits)

On the board stability: 1 month

Calibration Stability: 1 month

Make a new calibration when changing reagent batch, if quality control results are found out of the established range and after maintenance operations

Performances and stability data on KENZA 450TX/ISE and KENZA ONE are available on request.

QUALITY CONTROL

- REF 95010 EXATROL-N level I
- REF 95011 EXATROL-P level II
- REF 95012 Urinary controls
- External quality control program.

It is recommended to control in the following cases:

- At least once a run.
- At least once within 24 hours.
- When changing vial of reagent.
- After maintenance operations on the instrument.

If control is out of range, apply following actions:

1. Prepare a fresh control serum and repeat the test
2. If control is still out of range, use a new vial of fresh calibrator
3. If control is still out of range, use a new vial of reagent and reassay

If control is still out of range, please contact BIOLABO technical support or your local Agent.

I PROCEDURE

Manual method

Pipette into well identified test tubes:

	Reagent Blank	Specimen blank	Standard	Assay
Reagent	1 mL		1 mL	1 mL
Saline Solution		1 mL		
Demineralised water	20 µL			
Standard			20 µL	
Specimen		20 µL		20 µL

Mix well. Incubate for 2 minutes at room temperature.
Use a 1 cm path length cuvette and read Standard and assays absorbance at 340 nm (334-366) against Reagent blank.
Read Specimen blank against saline solution.

1. Urines:
   - Dilute specimen 1+9 in demineralized water
   - Use standard of the kit to calibrate (do not dilute)
   - Control with REF 95012 (to dilute as patient's urines)
2. Performances with manual procedure should be validated by user
3. KENZA applications and other applications proposal are available on request
4. Specimen blanking is recommended
5. Values of control sera and calibrator are indicated with or without specimen blank.

CALCULATION

Serum or plasma:

$$\text{Result} = \frac{\text{Abs (Assay)- Abs (Specimen blank)}}{\text{Abs (Standard) – Abs (Standard blank)}} \times \text{Standard concentration}$$

Urines diluted 1+9: Multiply the result by 10 (dilution factor).

REFERENCES

(1) *TIETZ N.W. Text book of clinical chemistry, 3rd Ed. C.A. Burtis, E.R. Ashwood, W.B. Saunders (1999) p. 1406-1457.*
(2) *Clinical Guide to Laboratory Test, 4th Ed., N.W. TIETZ (2006) p.852-855*
(3) *YOUNG D.S., Effect of Drugs on Clinical laboratory Tests, 4th Ed. (1995) p.3-456 to 3-462*
(4) *DALY J. A. and ERTINGSHAUSEN G.,Clin. Chem., Direct method for inorganic phosphate determination, (1972), 18, p.263-265*
(5) *GAMST O.K., TRY K., Scand. J. Clin. Lab. Invest. (1980), 40, p.483-486*
(6) *SRM: Standard Reference Material*