



INORGANIC PHOSPHOROUS

(LIQUID)

4 x 60 ml

RE – ORDER PHO1300

INTENDED USE:

This reagent is intended for the quantitative in vitro determination of inorganic phosphate in serum or urine.

CLINICAL SIGNIFICANCE:

Approximately 80% of phosphorus in the body is bound to calcium in bones and teeth. The remainder is distributed throughout the body in combination with lipids, carbohydrates, protein and other compounds. Serum inorganic phosphorus is increased following ingestion of carbohydrates, in children during summer months, following large doses of Vitamin D, ultraviolet irradiation, and during healing of fractures. In renal insufficiency, from many causes, elevation may occur. In severe nephritis elevations as high as 15 to 20 mg/dL are not uncommon. In rickets, phosphorus levels are low with values between 1 and 2 mg/dL. Other causes of low phosphorus are diseases in which there is a defective absorption.

TEST SUMMARY:

Phosphate reacts in acid medium with ammonium molybdate to form a phosphomolybdate complex with a yellow color. The intensity of this color is proportional to the concentration of inorganic phosphate in the sample; the color formed is measured at 340 nm (1).

The final color is formed within one minute and is stable for one hour. Interference is minimized by the use of a small sample and surfactants.

REAGENT COMPOSITION:

The concentration of the active ingredients in the reagents are:

Reactive ingredients:

Ammonium Molybdate 0.8 mmol/L

Non-reactive ingredients:

Surfactants, buffers, stabilizers and fillers

REAGENT PREPARATION:

The Phosphorus Reagent is liquid in ready to use form.

REAGENT STORAGE AND STABILITY:

The reagent is stable in the unopened bottles until the expiration date on the label when stored at room temperature (below 25 °C).

With time, the reagent may develop a blue color, particularly if stored for prolonged periods at room temperature. This does not interfere with the assay.

If the absorbance of the reagent at 340 nm is greater than 0.600 do not use the reagent.

PRECAUTIONS:

Harmful by inhalation, in contact with skin and if swallowed. Irritating to eyes and skin.

Avoid contact with skin and eyes. 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 for at least 10 minutes. If swallowed, seek medical advice immediately and show this container or label.

Good laboratory safety practices should be followed when handling any laboratory reagent. Refer to a recognized laboratory safety program for additional information. (See GP17-T, Clinical Laboratory Safety; Tentative Guideline (1994), National Committee on Clinical Laboratory Standards, Wayne, PA.)

Intended for in vitro diagnostic use only.

SPECIMEN COLLECTION, PREPARATION AND STORAGE:

Use only clear, unhemolyzed serum, separated from the erythrocytes as soon as possible. Erythrocytes contain organic phosphates which can hydrolyze on standing or can be enzymatically cleaved by phosphatases. Inorganic phosphates can then leak through the cell walls, increasing the concentration in serum (2).

Once the serum has been separated, the phosphate content will not change for at least a week when stored in the refrigerator (2–8 °C)(2).

Urine should be fresh. If alkaline, the urine should be acidified with concentrated hydrochloric acid to a pH of approximately 6. Store acidified

urine in the refrigerator (2–8 °C); reported stability is over 6 months (2).

Dilute urine 1:10 with water before assay.

INTERFERING SUBSTANCES:

Hemolysis renders serum unsuitable for assay. Glassware contaminated by detergents will affect the results.

Young, et al. (3) have published a comprehensive list of drugs and substances which may interfere with diagnostic assays, including inorganic phosphorus.

MATERIALS REQUIRED BUT NOT PROVIDED:

Spectrophotometer or colorimeter capable of measuring absorbance at 340 nm. Other wavelengths can be used from 340 to 360 nm.

Matched cuvettes.

Constant temperature incubator is not necessary; however, use the same temperature for assay of standard, controls and samples.

Distilled or deionized water.

Pipettes to measure water, reagent, standard and samples.

Standard or calibrator with an established value for phosphorus concentration.

MATERIALS PROVIDED:

Phosphorus Reagent in liquid form.

GLU1140 4 x 60 ML

TEST PROCEDURES:

Temperature need not be as closely regulated. However, perform the assay of the sample, standard and blank at the same temperature.

Wavelength: 340 nm

Temperature: 25 °C, 30 °C or 37 °C

Into a series of matched cuvettes, pipette:

Blank: 2 mL reagent + 20 µL water

Standard: 2 mL reagent + 20 µL standard

Sample: 2 mL reagent + 20 µL sample

Mix gently. Incubate at room temperature. The reaction is complete within one minute. The color is stable for one hour.

Set the instrument to 340 nm and adjust to zero absorbance with the reagent blank. Read the absorbance of the standard and sample.

CALIBRATION:

This assay requires the use of a phosphorus standard. Use Raichem Serum Calibrator (Cat. No. 85474), or other commercially available standards or calibrators.

QUALITY CONTROL:

Serum controls are recommended to monitor the performance of manual and automated assay procedures, providing a continued screening of the instrument, reagents and technique. Commercially available control material with established values for phosphorus concentration may be used.

CALCULATIONS:

A of Sample

————— × conc. of standard = mg/dL phosphorus

A of Standard

Sample calculation:

If the absorbance of the sample is 0.180, that of the standard is 0.470 and the concentration of the standard is 10 mg/dL, then:

0.180

————— × 10 = 3.8 mg/dL phosphorus

0.470

LIMITATIONS OF THE PROCEDURE:

Samples with phosphorus concentrations exceeding 30 mg/dL should be diluted with an equal volume of distilled or deionized water and the assay repeated; multiply the results by 2.

REAGENT PERFORMANCE:

Linearity: The assay is linear to 30 mg/dL phosphorus.

Correlation:

Serum: Results obtained with this reagent from 120 serum samples, some of which were fortified with organic phosphorus, ranging from 1.6 mg/dL to 29 mg/dL were compared with those obtained using the phosphorus reagent manufactured by Stanbio Laboratory, Inc. The correlation coefficient was 0.999 and the regression equation was $y = 0.994x + 0.209$.

Urine: Results obtained with this reagent from 65 diluted urine samples ranging from 0.07 gm/L to 3.0 gm/L were compared with those obtained using the phosphorus reagent manufactured by Stanbio Laboratory, Inc. The correlation coefficient was 0.999 and the regression equation was $y = 0.992x + 0.06$.

Precision: Serum

Within Run	Total					
Mean (mg/dL)	2.1	13.7	28.5	2.1	13.6	27.9
SD (mg/dL)	0.08	0.2	0.4	0.08	0.4	0.6
CV (%)	3.7	1.3	1.2	3.7	3.1	2.1
N	24	24	24	40	39	40

Sensitivity: Using a 1:100 sample to reagent ratio and reading at 340 nm, 10 mg/dL phosphorus will produce a net absorbance of approximately 0.470.

REFERENCE RANGE:

Adults: 2.5-4.8mg/dl

Children: 4.0-7.0mg/dl

Values are decreased during menstrual period and after meals. It is strongly recommended that each laboratory establish its own normal values.

REFERENCES:

Daly, J.A., Ertingshausen, G., Clin. Chem. 18: 263,1972.

Henry, R.J., Clinical Chemistry-Principles and Technics, New York, Harper and Row, 1974.

Young, D.S., Pestanec, L.C. and Gibberman, V, Clin. Chem. 21, 1D, 1975.

Manufactured For:
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