Intended Use
For **IN VITRO** quantitative determination of inorganic phosphorus in serum or plasma using manual or automated applications.

Clinical Significance
Measurements of IP are primarily used for diagnosing hypervitaminosis D, hypoparathyroidism, renal failure, rickets and Fanconi syndrome, as well as for monitoring the causes and treatments. (1)

Method History
Most of the Inorganic Phosphorus methods presented in the literature are based upon the reduction of a phosphomolybdate complex to molybdenum blue. In 1972 Daly and Ertingshausen (2) found that an unreduced phosphomolybdate complex absorbs ultraviolet light. Acidified ammonium molybdate with a non-ionic surfactant was used to measure serum Inorganic Phosphorus. Daly's procedure was modified and adapted for continuous flow instruments by Amador and Urban. (3) The Catachem Inorganic Phosphorus procedure is based upon the work of Amador and Urban.

Method Principle
Phosphorus from the serum sample mixes with the molybdate to form the phosphomolybdate complex. The increase in absorbance is monitored at 340nm. The reaction scheme illustrates the reaction that occurs in this method.

$$\text{Phosphorus + Ammonium Molybdate} \xrightarrow{H^+} \text{Phosphomolybdate Complex}$$

Reagent Content
The concentrations of the active ingredients in the reagents will be approximately as follows:

Inorganic Phosphorus Reagent

Water	
Ammonium Molybdate	0.30 mmol/L
Sulfuric Acid	0.14 mmol/L
Nonreactive ingredients	

Precautions
Avoid contact of reagent with skin and eyes. Should contact occur, wash affected area with plenty of cold water. **DO NOT PIPETTE REAGENTS BY MOUTH.**

Preparation Of Working Reagent
Catachem Inorganic Phosphorus Reagent is packaged in ready-to-use form. No preparation is required.

Reagent Storage And Stability
Store the Inorganic Phosphorus Reagent at room temperature or refrigerated. When stored as directed, the reagent is stable until the expiration date stated on the label.

Specimen Collection And Preparation
Test sera should be fresh, clear and unhemolyzed. When blood is drawn, it should be processed as soon as possible and the serum should be isolated from the clot without delay.

Precautions
Avoid contact of specimens with skin and eyes. Should contact occur, wash affected area with plenty of water. **DO NOT PIPETTE SPECIMENS BY MOUTH.**

Quality Control
To monitor the performance of the working reagent and the procedure used, we recommend the regular use of a normal and abnormal control serum.

Interfering Substances
Various substances have been reported to interfere with the inorganic phosphorus method. (4)

Expected Values
The range of expected values determined for this method in humans is 2.5 - 4.5 mg/dL. Values are species specific. It is recommended that each laboratory establish the normal range for the species under test and for the area in which the laboratory is located.

Procedure
Important: Read entire procedure instructions before proceeding with assay.

Materials Required (Not Provided)

Spectrophotometer	
Match Cuvettes	1 cm light path
Timer	to time incubation time
Pipette	2.0 ml for reagent
Pipette	0.04 ml for sample

Materials Provided
Inorganic Phosphorus Reagent

Analytical Parameters

Wavelength	340 nm
Pathlength	1 cm
Reaction Mode	endpoint
Reaction Time	5 minutes
Reagent Volume	1.0 ml
Sample Volume	0.015 ml
Total Volume	1.015 ml
Sample-to-reagent ratio	1:68

Note: To eliminate interferences for lipemic and icteric samples and to maximize accuracy in the assay procedure, all samples should be blank corrected.

Assay Procedures

1. Pipette 1.0 ml of IP reagent into each of three cuvettes marked “Calibrator”, “Sample”, and “Blank”.
2. Pipette 0.015 ml of calibrator or sample into their respective cuvettes. Use 0.015 ml of distilled water for the blank. Mix all cuvettes well.
3. Incubate all cuvettes for 5 minutes at room temperature.
4. Set spectrophotometer wavelength at 340 nm and zero the instrument with blank.
5. Read the “Calibrator” and “Sample” absorbencies.
6. Calculate the IP concentration (mg/dL) in the sample(s), as shown in calculations and results.

Blank Procedure

Follow the Assay Procedure and replace the IP Reagent with 0.9% Sodium Chloride.

Calculations And Results

$$\text{IP (mg/dL)} = \frac{\text{Sample Absorbance}}{\text{Calibrator Absorbance}} \times \text{Calibrator (mg/dL)}$$

Example:

	Assay OD	Blank OD
Sample	0.320	0.020
Calibrator	0.250	0.015

Calibrator = 5.0 mg/dL

$$\text{IP (mg/dL)} = \frac{0.320 - 0.020}{0.250 - 0.015} \times 5 \text{ mg/dL}$$

= 6.4 mg/dL

Method Performance Characteristics

Sensitivity: 0.040-0.051 absorbance units per mg/dL.
Linear Range: 0-10 mg/dL.
Precision: Within-run and day-to-day precision is summarized below.

Precision Study

I. P.	Within-Run Precision		Total Precision	
Mean	SD	CV	SD	CV
Mg/dL	Mg/dL	%	Mg/dL	%
1.7	0.11	6.2	0.14	8.5
5.2	0.16	3.0	0.18	3.4
9.0	0.16	1.7	0.30	1.7

Correlation

A comparison of this method using an automated analyzer and reference method based upon the molybdate reaction resulted in the following regression statistics:

Range	=	1.6-7.3 mg/dL
N	=	196
Y	=	0.981x + 0.08
r	=	0.990
Sy.x	=	0.15

References

1. Fundamentals of Clinical Chemistry, Edited by Norbert Teitz. WB Saunders, Philadelphia (1976).
2. Daly JA and Ertinghausen G. Direct method for determining inorganic phosphate in serum with the CentrifiChem. Clin Chem 18, 263 (1972).
3. Amador E and Urban J. Simplified serum phosphorus analysis by continuous flow ultraviolet spectrophotometry. Clin Chem Vol 18 No 7, 601 (1972).
4. Young DS, Pestaner LC, Gibberman V: Effects of drugs on clinical laboratory tests. Clin Chem 21(5):1D-432D (1975).
5. Martin EW. Hazards of Medication (Alexander SF, Farage DJ and Hassan WE Jr. Eds) Philadelphia, PA and Toronto, Canada. JB Lippincott Co (1971) pp169-189.
6. Constantino NV, Kabat HF. Drug induced modifications of laboratory test values revised 1973. Am J Hosp Pharm 30:24-71 (1973).

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353 Christian Street, Suite #2, Oxford, Connecticut 06478 USA ● Phone (203) 262-0330 ● Fax (203) 262-9836 ● email: catachem@catacheminc.com