Pointe Inorganic Phosphorus Reagent Set (UV)

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Intended Use
For the quantitative determination of Inorganic Phosphorus in serum using the Yumizen C230 and Yumizen C240 analyzers. **Rx Only.**

Method History
The measurement of inorganic phosphorus in serum is usually accomplished by forming a phosphomolybdate complex and in turn reducing it to a molybdenum blue color complex. Methods differ as to the choice of reducing agents: stannous chloride[1], phenylhydrazine[2], aminonaphtholsulfonic acid[3], ascorbic acid[4], ρ-methylaminophenolsulfate[5], N-phenyl-ρ-phenylenediamine[6] and ferrous sulfate.[7] These methods suffered from color instability, deproteinization steps and complexity of performance[8]. The addition of a surfactant eliminated the need to prepare a protein-free filtrate, accelerated color production, stabilized the color and simplified the procedure. Many of the components in these reagents were unstable and had to be stored separately. The quantitative measurement of unreduced phosphomolybdate complexes was first reported by Simonsen in 1946.[9] Daly and Ertingshausen[10] adapted that technique for the determination of inorganic phosphorus in 1972. Amador and Urban[11] modified this procedure further the same year. The present method is a modification of the above procedure using a single, stable reagent performing in the UV range.

Principle
Inorganic Phosphorus + H_2SO_4 + Ammonium Molybdate ---------➢ Unreduced Phosphomolybdate Complex

Inorganic phosphorus reacts with ammonium molybdate in an acid medium to form a phosphomolybdate complex that absorbs light at 340nm. The absorbance at this wavelength is directly proportional to the amount of inorganic phosphorus present in the sample.

Reagents
Ammonium Molybdate 0.48 mM, Sulfuric Acid 220 mM with surfactant

Precautions
1. This reagent is for *in vitro* diagnostic use only.
2. This reagent is an acid and is caustic. Avoid contact with skin. Flush with plenty of water if contact occurs. DO NOT PIPETTE BY MOUTH.

Reagent Preparation
Reagent comes in a ready to use form.

Reagent Storage
Store reagent at refrigerator temperature(2-8°C). The reagent is stable until the expiration date appearing on the label when stored as directed.

Reagent Deterioration
Do not use reagent if:
1. Reagent read against water has an absorbance greater than 0.500 at 340 nm.
2. The reagent fails to recover stated control values.

Specimen Collection and Storage
1. Unhemolyzed serum is specimen of choice.
2. Plasma should not be used since anticoagulants may produce falsely low values.[12]
3. Hemolyzed sample may give falsely high values.
4. Serum should be removed from the red cell clot as soon as possible.[13]
5. Serum inorganic phosphorus is stable for one week refrigerated and for three weeks frozen.[13,14]

Interferences
For a comprehensive list of substances that interfere with the measurement of Inorganic Phosphorus see Young, et al.[15]

Materials Provided
Inorganic Phosphorus Reagent

Materials Required but not Provided
1. Yumizen C230 / Yumizen C240 Analyzer
2. Yumizen C230 / Yumizen C240 Operation Manual
3. Pointe Chemistry Calibrator, catalog number C7506-50
4. Pointe Chemistry control, catalog number C7592-100

Test Parameters

Test:	PHOS	Chemistry:	Phosphorus
Chemistry No.:	227	Print Name:	PHOS
Reaction Type:	Endpoint	Reaction Direction:	Positive
Pri. Wave:	340 nm	Sec. Wave	
Decimal.:	0.1	Samp. Type:	Serum
Blank Time:		Reaction Time:	7 8
Unit:	mg/dL	Incubation Time:	0

	Sample Vol.	Aspirated	Diluent		Reagent Vol.	Diluent	
Standard;	2	uL	uL	uL	200	uL	uL
Decreased;		uL	uL	uL			
Increased;		uL	uL	uL			

Linearity Range (Standard);	0-12		Linearity Limit:		
Linearity Range (Decreased):			Substrate Depletion:		
Linearity Range (Increased):			Mixed Blank Abs.:	- 40000	40000
R1 Blank Abs.:	- 40000	40000	On-board Stability:	30	Day (s)
Blank Response	- 40000	40000	Reagent Alarm Limit:	5	
Twin Chemistry:					

Prozone Check:		
Q1:	Q2:	Q3:
Q4:	PC:	ABS:

Use Qualitative Result:		
	Range:	Flag:

Slope Offset:			
	Slope	Offset	Unit
	1	0	mg/dL

Pretreatment:			
Pretreat Sample Vol.:	uL	Pretreat Reagent Vol.:	uL

Ref. Range:					
Sample Type:	Gender:	Age Range:	Ref. Range:	Critical Range:	Unit:

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Calibration Setup Parameters

Chem: PHOS
Calibration Setting
Math Model: Two-Point Linear
Factor: Replicates: 2
Acceptance Limits
Cal Time: 336 hr.
Slope Diff: SD:
Sensitivity: Repeatability: * User Defined
Deter Coeff:
Auto Calib.
☐ Cal Time

Calibrator	Conc.	Pos	Lot No.
Water	0.0	W	
Chem Cal	*	*	

Calibration

Use an NIST-traceable serum calibrator. The procedure should be calibrated according to the instrument manufacturer's calibration instructions. If control results are found to be out of range, the procedure should be re-calibrated.

Quality Control

The integrity of the reaction should be monitored by use of normal and abnormal control sera with known concentrations of inorganic phosphorus. Quality control requirements should be performed in conformance with local, state, and/or Federal regulations or accreditation requirements.

Calculation (Example)

Abs. = Absorbance

$$\frac{\text{Abs. of Unknown}}{\text{Abs. of Standard}} \times \text{Concentration of Standard} = \text{Inorganic Phosphorus (mg/dl)}$$

Example: Abs. of Unknown = 0.20; Abs. of Standard = 0.29; Conc. of Standard = 5 mg/dl

Then: $\frac{0.20}{0.29} \times 5 = 3.4$ mg/dl

SI Units

To obtain results in SI Units (mmol/L), multiply the results in mg/dl by the factor 0.323.

Example: 3.4 mg/dl x 0.323 = 1.09 mmol/L.

Limitations

Detergents containing phosphate should not be used for cleaning glassware used in this procedure.

Expected Values

Adults: 2.5-4.8mg/dl[16]
Children: 4.0-7.0mg/dl[17]

Values are decreased during menstrual period and after meals.[17]
It is strongly recommended that each laboratory establish its own normal values.

Performance

1. Linearity: 12 mg/dl
2. Comparison: A study was performed between the Yumizen 200 series analyzers and a similar analyzer and method, resulting in a correlation coefficient of 0.994 with a regression equation of y = 0.902x + 0.07 (N=37).
3. Precision: Precision studies were performed using the Yumizen 200 series analyzers following a modification of the guidelines which are contained in NCCLS document EP5-T2.[18]

Within Run			Run to Run		
Mean	**S.D.**	**C.V.%**	**Mean**	**S.D.**	**C.V.%**
3.21	0.12	3.8	3.54	0.07	1.98
7.17	0.21	3.0	7.99	0.20	2.50

References

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Symbol Key

Use by (YYYY-MM-DD)	LOT Lot and batch code
REF Catalog number	Manufacturer
IVD *In vitro* diagnostic medical device	Temperature limitation
Consult instructions for use	**Rx Only:** Prescription Use Only
CE mark	EC REP Authorized representative in the European Community

REF 12-P7516-160

Manufactured by
HORIBA Instruments Incorporated - Pointe Brand
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IVD

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Certified to Perform Reagents

The Pointe reagents are certified to be manufactured according to specified parameters. Any Pointe reagent product not meeting specifications through its listed expiration date will be remedied immediately without charge.

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