Pointe Inorganic Phosphorus Reagent Set (UV)

HORIBA Instruments Incorporated
5449 Research Drive, Canton, MI 48188
Phone: 734-487-8300; (800) 445-9853

Intended Use
For the quantitative determination of Inorganic Phosphorus in serum using the Yumizen C560 analyzer. **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], p-methylaminophenolsulfate[5], N-phenyl-p-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 and Hazards
This reagent is for *in vitro* diagnostic use only.

Hazards:
Hazard Classifications: Skin Corrosion/Irritation (Category 1), Serious eye damage/eye irritation (Category 1), Specific target organ toxicity, single exposure; Respiratory System (Category 1), Specific target organ toxicity, repeat exposure; Respiratory System (Category 1)
Hazard Statements: H314: Causes severe skin burns and eye damage, H319: Causes serious eye damage, H370: Causes damage to organs, H372: Causes damage to organs through prolonged or repeated exposure

Signal Word: Danger

Precautionary Statements
Prevention : P260: Do not breathe dust/fume/gas/mist/vapors/spray, P264: Wash hands thoroughly after handling, P270: Do not eat, drink or smoke when using this product, P280: Wear protective gloves/protective clothing/eye protection/face protection
Response : P314: Get medical advice/attention if you feel unwell, P363: Wash contaminated clothing before reuse, P301 + P330 + P331: IF SWALLOWED: Rinse mouth. Do NOT induce vomiting, P303 + P361 + P353: IF ON SKIN (or hair): remove/Take off immediately all contaminated clothing. Rinse SKIN with water/shower, P304 + P340: IF INHALED: Remove victim to fresh air and Keep at rest in a position comfortable for breathing, P305 + P351 + P338: IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing, P307 + P311: IF exposed: call a POISON CENTER or doctor/physician.
Storage : P404 : Store in a closed container.
Disposal : P501 : Dispose of contents into sewer system after diluting with large volumes of water, if in accordance with local regulations. **Refer to the Safety Data Sheet for this product (SDS-P7516) available by calling 1-734-487-8300.**

Reagent Preparation
Reagent comes in a ready to use form.

Reagent Storage and Stability
Store reagent at refrigerator temperature (2-8°C). The reagent is stable until the expiration date appearing on the label when stored as directed. Manufacturer studies have shown reagent is stable for 30 days once placed in the refrigerated reagent carousel (2-10°C), however reagent stability may vary based on individual laboratory conditions.

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]

Pointe Inorganic Phosphorus Reagent Set (UV)

Materials Provided
Inorganic Phosphorus Reagent

Materials Required but not Provided
1. Yumizen C560 Analyzer
2. Yumizen C560 operation manual
3. Chemistry Calibrator, catalog number C7506-50
4. Chemistry control, catalog number C7592-100

Calibration
Use Pointe Chemistry Calibrator (Catalog Number C7506-50). The procedure should be calibrated according to the instrument manufacturer's calibration instructions. If control results are found to be out of range, the test may need to be re-calibrated. Under typical operating conditions manufacturer calibration stability studies have shown the calibration curve will be stable for at least 14 days.

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.

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. Lipemic and icteric samples require a serum blank. For maximum accuracy a serum blank should be run with each sample.

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. Assay Range: 0.0-12.0 mg/dL.
2. Correlation: A study was performed between the Yumizen C560 and a similar analyzer using this method, resulting in the following:

Method	Phosphorus
N	96
Mean Phosphorus (mg/dL)	4.30
Range (mg/dL)	0.5-9.9
Standard Deviation	1.83
Regression Analysis	y = 0.936x + 0.25
Correlation Coefficient	0.9724

3. Precision: Precision studies were performed following a modification of the guidelines contained in the NCCLS document EP5-T2.[18]

Within Day

Sample	LOW	MID	HIGH
N	20	20	20
Mean	3.78	7.31	10.89
Standard Deviation	0.04	0.04	0.04
Coefficient of Variation (%)	1.1%	0.6%	0.4%

Total

Sample	LOW	MID	HIGH
N	40	40	40
Mean	3.81	7.38	11.06
Standard Deviation	0.13	0.31	0.31
Coefficient of Variation (%)	3.5%	4.2%	2.8%

4. Sensitivity: 2SD limit of detection (95% Conf) = 0.0 mg/dL

References

1. Osmond, M.F., Bull. Soc. Chim. 47:745 (1887).
2. Taylor, A.E., Miller, C.W., J. Biol., Chem 18:215 (1914).
3. Fiske, C.H., Subbarow, Y., J. Biol. Chem. 66:275 (1925).
4. Lowry, O.H., Lopez, J.A., J. Biol. Chem. 162:421 (1946).
5. Power, M.H., Standard Methods of Clinical Chemistry New York, Academic Press, (1953).
6. Dryer, R.L., et al, J. Biol. Chem. 225:177 (1957).
7. Taussky, H.H., Shorr, E., J. Biol. Chem. 202:675 (1953).
8. Martinek, R.G., J. Am. Med. Tech. 32:337 (1970).
9. Simonsen, D.G., et al, J. Biol. Chem. 166:747 (1946).
10. Daly, J.A., Ertingshausen, G., Clin. Chem. 18:263 (1972).
11. Amador, E., Urban, J., Clin. Chem. 18:601 (1972).
12. Goldenberg, H. Ferrnandez, A. Clin. Chem. 12:871 (1966).
13. Henry, R.J., et al, Clinical Chemisty: Principles and Technics, New York, Harper & Row, pp.122:143 (1964).
14. Hansk, A., Kao, J., Clin. Chem. 14:58 (1968).
15. Young, D.S., et al, Clin. Chem., 21:1D, (1975).

HORIBA Instruments Incorporated
5449 Research Drive, Canton, MI 48188
Phone: 734-487-8300; (800) 445-9853
HORIBA Medical

16. Henry, R.J., et al, Clinical Chemistry: Principles and Technics, 2nd Ed., Hagerstown (MD), Harper & Row, p.728 (1974).
17. Tietz, N.W., Fundamentals of Clinical Chemistry, Philadelphia, W.B. Saunders, p.917 (1976).
18. NCCLS document "Evaluation of Precision Performance of Clinical Chemistry Devices", 2nd Ed. (1992).

CHEMISTRY PARAMETERS

Chem:	PHOS	No.:	227	Sample Type:	Serum
Chemistry:	Inorganic Phosphorus			Print Name:	PHOS
Reaction Type:	End Point			Reaction Direction:	Positive
Pri Wave:	340			Sec Wave:	
Unit:	mg/dL			Decimal	0.1
Blank Time:	10	12		Reaction Time:	27 29

	Sample Vol.	Aspirated	Diluent	Reagent Vol.	Diluent
Standard:	1.5 ul	--- ul	--- ul	R1: 150 ul	--- ul
Decreased:	--- ul	--- ul	--- ul	R2: --- ul	-- ul
Increased:	--- ul	--- ul	--- ul	R3: --- ul	-- ul
	☐ Sample Blank	☑ Auto Rerun		R4: --- ul	--- ul

Slope/Offset Adjustment

Slope: 1 Offset: 0

Linearity Range (Standard)	0	12		Linearity Limit:
Linearity Range (Decreased)	____	____		Substrate Depletion:
Linearity Range (Increased)	____	____		Mixed Blank Abs:
R1 Blank Abs:	____	____		Uncapping Time
Blank Response:	____	____		Reagent Alarm Limit:
Twin Chemistry:				☐ Enzyme Linear Extension

☐ Prozone Check ○ Rate Check ○ Antigen Addition

Q1: Q2: Q3: Q4:

PC: ABS:

Pointe Inorganic Phosphorus Reagent Set (UV)

CALIBRATION PARAMETERS

Calibrator Definition

Calibrator:	*	Lot No.:	*
Exp Date:	*		

Carousel	**Pos**
Sample Carousel 1	*
Sample Carousel 2	
Sample Carousel 3	

Reagent/Calibration

Calibrator	Pos	Lot No	Exp Date	Chem	Conc	Unit
Water	W	*	*	PHOS	0	mg/dL
Chemistry Calibrator	*	*	*	PHOS	*	mg/dL

Calibration Setup

Chem:	PHOS		
Calibration Settings			
Math Model:	Two-Point Linear		
Factor:		Replicates:	2
Acceptance Limits			
Cal Time:	336	Hour	
Slope Diff:	---	SD:	---
Sensitivity :	---	Repeatability:	---
Deter Coeff:	---		
Auto Calib.			

☐ Bottle Changed ☐ Lot Changed ☐ Cal Time

It is recommended that two levels of control material be assayed daily.
* Indicates user defined parameter.

REF 14-P7516-174

Manufactured by
HORIBA Instruments Incorporated-Pointe Brand
5449 Research Drive Canton, MI 48188

2°C 8°C IVD

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.

Manufactured by HORIBA Instruments Incorporated – Pointe Brand
5449 Research Drive, Canton, MI 48188

European Authorized Representative:
Obelis s.a.
Boulevard Général Wahis 53
1030 Brussels, BELGIUM
Tel: (32)2.732.59.54 Fax:(32)2.732.60.03 email: mail@obelis.net

CE

Symbol Key

Use by (YYYY-MM-DD) LOT Lot and batch code REF Catalog number
Manufacturer Temperature limitation Consult instructions for use
IVD *In vitro* diagnostic medical device **Rx Only:** Prescription Use Only