Inorganic Phosphorus Reagent
Direct UV Method Without Reduction

PRODUCT SUMMARY

Stability	:	Until Expiry at 2-25°C
Linear Range	:	0.0 - 5.00 mmol/L (0 - 15.0 mg/dL)
Specimen Type	:	Serum, plasma or urine
Method	:	Endpoint
Reagent Preparation	:	Supplied ready to use.

IVD

SYMBOLS IN PRODUCT LABELLING

EC REP	Authorized Representative		Temperature Limitation
IVD	For in vitro diagnostic use		Use by/Expiration Date
LOT	Batch code/Lot number		CAUTION. CONSULT INSTRUCTIONS FOR USE.
REF	Catalogue number		Manufactured by
	Consult instructions for use		Xi - Irritant

INTENDED USE
This reagent is intended for the in vitro quantitative determination of Inorganic Phosphorus in human serum, plasma or urine.

CLINICAL SIGNIFICANCE
More than 80% of the body's phosphorus is present in bones as calcium phosphate. The remainder is found intracellularly as organic phosphates such as phospholipids, nucleic acids and ATP or extracellularly as inorganic phosphorus.
There is generally a reciprocal relationship between serum calcium and inorganic phosphate levels. Increased levels of serum phosphorus are seen in renal disease, hypo-parathyroidism and excessive Vitamin D intake. Decreased levels are seen in rickets, osteomalacia (adult rickets), hyperparathyroidism and diabetic coma. [1]

METHODOLOGY
Most methods for inorganic phosphate in serum involve the formation of phosphomolybdate and subsequent reduction to molybdenum blue. Problems with variable color formation and reagent instability are common to these methods. [2,3]
This procedure is based on the method of Daly and Ertingshausen[4] as modified by Wang, et al. [5] The formation of the unreduced phosphomolybdate is measured at 340 nm and is directly proportional to the amount of inorganic phosphate present.

REAGENT COMPOSITION

Active Ingredient	Concentration
Ammonium molybdate	0.8 mmol/L
Sulfuric Acid	430 mmol/L
Sodium chloride	77 mmol/L

pH < 1.0 at 20°C.

WARNING: Do not ingest. If spilt, thoroughly wash affected areas with water. Flush with plenty of water when disposing. For further information consult the Inorganic Phosphorus reagent Material Safety Data Sheet.

R38	Irritating to skin.
R41	Risk of serious damage to eyes.
S24/25	Avoid contact with skin and eyes.

REAGENT PREPARATION
Reagent is supplied ready to use.

STABILITY AND STORAGE
Prior to use:
When stored between 2-25°C and protected from light, the reagent is stable until the expiration date stated on the bottle and kit box label.

Once the reagent is opened:
When stored capped between 2-25°C and protected from light, the reagent is stable until expiry.

Indications of Reagent Deterioration:
- Turbidity;
- Reagent Absorbance > 0.5 AU at 340 nm; and/or
- Failure to recover control values within the assigned range.

SPECIMEN COLLECTION AND HANDLING[2]
Serum: Use non-haemolysed serum.
Plasma: Use heparin.
Urine: Accurate analysis of Urine phosphate can only beperformed if all precipitated phosphate is dissolved prior to testing. Twenty Four hour urine specimens should be acidified with 15 mL of concentrated HCl. Non acidified specimens which have been refrigerated should be acidified and/or heated at 56°C for 15 minutes to redissolve any precipitate. (Acidified specimens are unsuitable for urate or creatinine estimations). Dilute urine specimens 1 in 20 with distilled or deionized water prior to analysis.
Storage: Serum or plasma samples are stable for 7 days at room temperature (18-25°C) or 4°C and for 6 months frozen[1]. Urine samples when stored at 4°C are stable for 7 days. [6]

ADDITIONAL EQUIPMENT REQUIRED BUT NOT PROVIDED
- If required, pipettes for accurately dispensing measured volumes.
- A clinical chemistry analyser capable of maintaining constant temperature (37°C) and measuring absorbance at 340 nm.
- Analyser specific consumables, eg: sample cups.
- Normal and abnormal assayed control material.
- Calibrator or a suitable aqueous Inorganic Phosphorus standard.

ASSAY PROCEDURE
The following system parameters are recommended. Individual instrument applications are available upon request from the Technical Support Group.

SYSTEM PARAMETERS

Temperature	30°C / 37°C
Primary Wavelength	340 nm
Secondary Wavelength	380 nm
Assay Type	Endpoint
Direction	Increase
Sample : Reagent Ratio	1 : 50
eg: Sample Vol	3µL
Reagent Vol	150µL
Incubation Time	6 minutes
Reagent Blank Limits	Low 0.0 AU
(340nm, 1cm lightpath)	High 0.5 AU
Linearity	0.0-5.00 mmol/L (0-15.0 mg/dL)
Analytical Sensitivity	0.357 ΔA per mmol/L
(340nm, 1cm lightpath)	0.115 ΔA per mg/dL

CALCULATIONS
Results are calculated, usually automatically by the instrument as follows:

$$\text{Inorganic Phosphorus} = \frac{\text{Absorbance of Unknown}}{\text{Absorbance of Calibrator}} \times \text{Calibrator Value}$$

Example:
Absorbance of calibrator = 0.496
Absorbance of unknown = 0.387
Value of calibrator = 1.39 mmol/L (4.31 mg/dL)

$$\text{Inorganic Phosphorus} = \frac{0.387}{0.496} \times 1.39 = 1.08 \text{ mmol/L}$$

$$\text{Inorganic Phosphorus} = \frac{0.387}{0.496} \times 4.31 = 3.36 \text{ mg/dL}$$

To convert the urinary phosphorus result from mmol/L to mmol/24 hours, the following formula should be used:-

$$\text{Urine Phosphorus (mmol/24 hours)} = \text{Phosphorus Result (mmol/L)} \times \text{Dilution Factor} \times \text{Volume (L)}$$

Thermo SCIENTIFIC

Example:

Phosphorus	=	1.55 mmol/L (4.81 mg/dL)		
Dilution	=	1 : 20		
Vol of Urine	=	1.25 L (12.5 dL)		
Urine Phosphorus	=	1.55 x 20 x 1.25	=	38.8 mmol/24 Hours
Urine Phosphorus	=	4.81 x 20 x 12.5	=	1203 mg/24 Hours

NOTES

1. The sample and reagent volumes may be altered proportionally to accommodate different spectrophotometer requirements.
2. Specimens with inorganic phosphorus values greater than 5.00 mmol/L (15.0 mg/dL) should be diluted and reassayed. Multiply the results by the dilution factor.
3. The color development is stable for at least 1 hour.
4. Unit conversion: mmol/L x 3.1 = mg/dL.

CALIBRATION

Calibration is required. An aqueous standard or serum based calibrator, with an assigned value traceable to a primary standard (eg. NIST or IRMM) is recommended. For calibration frequency on automated instruments, refer to the instrument manufacturer's specifications. However, calibration stability is contingent upon optimum instrument performance and the use of reagents which have been stored as recommended in the stability and storage section of this package insert. Recalibration is recommended at anytime if one of the following events occurs:-

- The lot number of reagent changes.
- Preventative maintenance is performed or a critical component is replaced.
- Control values have shifted or are out of range and a new vial of control does not rectify the problem.

QUALITY CONTROL

To ensure adequate quality control, normal and abnormal controls should be run as unknown samples:-

- At least once per day or as established by the laboratory.
- When a new bottle of reagent is used.
- After preventative maintenance is performed or a critical component is replaced.
- With every calibration.

Control results falling outside the upper or lower limits of the established ranges indicate the assay may be out of control.

The following corrective actions are recommended in such situations:-

- Repeat the same controls.
- If repeated control results are outside the limits, prepare fresh control serum and repeat the test.
- If results are still out of control, recalibrate with fresh calibrator, then repeat the test.
- If results are still out of control, perform a calibration with fresh reagent, then repeat the test.
- If results are still out of control, contact Technical Services or the local distributor.

LIMITATIONS

1. Studies to determine the levels of interference from haemoglobin, bilirubin and lipaemia were carried out. The following results were obtained:
   Haemoglobin: No interference from haemoglobin up to 160 mg/dL.
   Bilirubin: No interference from bilirubin up to 340 µmol/L (20 mg/dL).
   Lipaemia: No interference from lipemia, measured as triglycerides, up to 5.1 mmol/L (450 mg/dL).
2. Young[7] has published a comprehensive list of drugs and substances which may interfere with this assay.

EXPECTED VALUES[8]

Serum:	0.8 - 1.5 mmol/L	(2.5 - 4.5 mg/dL)
24 hour Urine:	13 - 42 mmol/24 hr	(0.4 - 1.3 g/24 hr)

The quoted values should serve as a guide only. It is recommended that each laboratory verify this range or derive a reference interval for the population it serves. [9]

PERFORMANCE DATA

The following data was obtained using the Inorganic Phosphorus reagent on a well maintained automated clinical chemistry analyser. Users should establish product performance on their specific analyser used.

IMPRECISION

Imprecision was evaluated over a period of 20 days using two levels of commercial control and following the NCCLS EP5-T procedure.[10]

		LEVEL I	LEVEL II
	Number of data points	80	80
	Mean (mmol/L / mg/dL)	0.70 / 2.17	1.04 / 3.22
Within Run:	SD (mmol/L / mg/dL)	0.05 / 0.16	0.06 / 0.19
	CV (%)	2.3	1.8
Total:	SD (mmol/L / mg/dL)	0.13 / 0.40	0.24 / 0.74
	CV (%)	6.2	7.5

METHOD COMPARISON

Comparison studies were carried out using a similar commercially available Inorganic Phosphorus reagent as a reference. Serum, plasma (Heparin) and urine samples were assayed in parallel and the results compared by least squares regression. The following statistics were obtained:

Serum/Plasma:

Number of sample pairs	40
Range of sample results	0.66 - 2.56 mmol/L (2.05 - 7.95 mg/dL)
Mean of reference method results	1.23 mmol/L (3.8 mg/dL)
Mean of Inorganic Phosphorus results	1.19 mmol/L (3.7 mg/dL)
Slope	0.91
Intercept	0.07 mmol/L (0.2 mg/dL)
Correlation coefficient	1.00

Urine:

Number of sample pairs	44
Range of sample results	2.9-62.6 mmol/L (9.0-194.0 mg/dL)
Slope	1.008
Intercept	0.32 mmol/L (1.0 mg/dL)
Correlation coefficient	0.9995

LINEARITY

When run as recommended the assay is linear between 0 and 5.00 mmol/L (0 - 15.0 mg/dL).

ANALYTICAL SENSITIVITY

When run as recommended the sensitivity of this assay is 0.357 ΔAbs per mmol/L or 0.115 ΔAbs per mg/dL (1 cm light path, 340nm).

REFERENCES

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Fisher Diagnostics
a division of Fisher Scientific Company, LLC
a part of Thermo Fisher Scientific Inc.
Middletown, VA 22645-1905 USA
Phone: 800-528-0494
540-869-3200
Fax: 540-869-8132

EC REP MDCI Ltd.
Arundel House
1 Liverpool Gardens
Worthing, West Sussex BN11 1SL UK

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REF

Reorder Information

Catalogue No.	Configuration
TR30026	2 x 250 mL