

INORGANIC PHOSPHOROUS

COLORIMETRIC METHOD

Principle:(1,2)

Inorganic phosphate in serum reacts with molybdic acid to form a phosphomolybdic acid complex, which is reduced by ammonium iron (II) sulphate to molybdenum blue, which is measured at 690 nm.

Reagent Concentrations:

Buffer	Molybdate Reagent	4g/l
	Sulphuric Acid	0.43M
Reductant	Ammonium Sulphate	72g/l
Diluent	Sulphuric Acid	0.43M
	Surfactant	10g/l
Standard	Phosphorous	1.61mmol/l

Preparation and stability of Reagents:

1. Molybdate

Contents ready for use. Stable up to expiry date when stored at +15 to +25 °C

2. Reductant

Dissolve the contents of one vial of Reductant in an aliquot from a full bottle of Reductant Diluent. Quantitatively transfer the contents of the Reductant bottle to the Diluent bottle, rinsing the vial several times. Stable 14 days at +2 to +8 °C, 7 days at +15 to +25 °C, and 2 months at -20 °C.

3. Standard

Mix together equal volumes of Molybdate 1 and Reductant 2. Stable for 3 days at +15 to +25 °C.

Working reagent:

Mix together equal volumes of Molybdate 1 and Reductant 2. Stable for 3 days at +15 to +25 °C.

Sample:

Serum or plasma. Avoid highly turbid samples.

Procedure:

Wavelength: 690nm
 Temperature: +20 to +25°C
 Cuvette: 1cm light path
 Zero adjustment: Against reagent blank
 Pipette into test-tubes:

	Reagent Blank	Standard	Sample
Working Reagent	1.0ml	1.0ml	1.0ml
Deionized water	30 TI	---	---
Standard	---	30 TI	---
Sample	---	---	30TI

Mix, let stand for 10 min at 25 °C. Read absorbance of sample (A sample) and standard (A standard) against reagent blank.

Calculation:

Conc. of inorganic phosphorous =

$$\frac{? \text{ A sample}}{? \text{ A standard}} \times \text{concentration of standard}$$

Normal values in Serum (3):

0.8 – 1.6mmol/l (2.5 – 4.97 mg/dl)

Quality control:

For accuracy and reproducibility control:-

Assayed Multi-Sera Normal and Elevated.

For reproducibility control:-

Multi-Sera Loaw, Normal and Elevated.

Linearity:

The method is linear to 0.8 mmol/l (24.8 mg/dl). Samples with higher concentrations should be diluted 1+1 with 0.9% NaCl solution and reassayed. Multiply the result by 2.

Presentation:

INP0330	2x125ml	1. Molybdate reagent	1 x 125 ml
		2. Reductant	1 vial
		3. Reductant diluent	1 x 125 ml
		4. Standard	1 x 5 ml

Safety precautions:

For in vitro diagnostic use only. Do not pipette by mouth. Exercise the normal precautions required for handling laboratory reagents.

Health and Safety data sheets are available on request.

References:

- 1) Taussky H., et al., J. Biol. Chem. 1953: **202**: 675.
- 2) Goldenberg H., et al., Clin Chem. 1966; **12**: 871.
- 3) Clinical Chemistry Principles and Technics nd Edition 1974. Eds. Henry Cannon, and Winkelman. Harper and Row Publishers, New York.