



## PHOSPHOROUS KIT (UV METHOD)

### PRINCIPLE:

Phosphorous reacts with ammonium molybdate in an acid medium to form a phospho molybdate complex which absorbs light at 340 nm. The absorbance at this wavelength is directly proportional to concentration of Phosphorous present in the sample.

### REAGENT PREPARATION & STABILITY:

1. Reagent R1 :Ready -to-Use
2. Phosphorous Standard (5 mg/dl) :Ready-to- Use

The reagents are stable until the expiry date mentioned on the label.

### SPECIMEN COLLECTION AND STORAGE

1. Use only clear, unhemolyzed serum, separated from the erythrocytes as soon as possible. Erythrocytes contain organic phosphates which can hydrolyze on standing or can be enzymatically cleaved by Phosphatase. Phosphates can then leak through the cell walls, increasing the concentration in serum.
2. Once the serum has been separated, the Phosphate content will not change for at least a week when stored at 2<sup>o</sup>-8<sup>o</sup>C.

### PROCEDURE:

WAVE LENGTH	: 340 nm	
TEMPERATURE	: Room Temperature	
CUVETTE	: 10 nm path length	
MEASUREMENT	: Reagent Blank	
INCUBATION	: 5 mins.	
STANDARD	: 5 mg/dl	
Pipette into cuvettes	Macro	Semi-Micro
Reagent R1	1000 ul	500 ul
Sample /Standard	20 ul	10 ul

Mix & read the absorbance of the sample (A sam.) and Standard (A Std.) against reagent blank after 1 min. at 340 nm.

**NOTE:** Serum Phosphate is stable for one week at 2<sup>o</sup>-8<sup>o</sup>C.

Most commonly employed detergents contain phosphates. Use of improperly rinsed glassware may result in elevated phosphorous values. Disposable plastic tubes or dilute Hydrochloric acid-washed glass tubes/containers should be used.

### CALCULATIONS:

$$\frac{(A2 - A1) \text{ of Sample}}{(A2 - A1) \text{ of Standard}} \times \text{Concentration of Standard} = \text{Phosphorous (mg/dl)}$$

To obtain results in mmol/l, multiply the results in mg/dl by the factor 0.323

EXPECTED VALUES	Women : 1.6 - 6.8 mg/dl
	Men : 2.1 - 5.6 mg/dl
	Children : 4.0 - 7.00 mg/dl
	Urine : 0.3 - 1.00 g/24h

LINEARITY : 12 mg/dl

### REFERENCES:

1. Tietz, N.W., Fundamentals of Clinical Chemistry.p.903, W.B. Saunders Co., Philadelphia, 1976.
2. Young, D.S.:et al., Clin. Chem, 21, 5 (1975).
3. Daly J.A. Ertingshausen G., Clin. Chem. 18, 263 (1972)