

# PHOSPHOLIPIDS

**Colorimetric Method**

**50 Tests**

## PRINCIPLE :

Phospholipids are precipitated with trichloroacetic acid and oxidized to phosphate with sulphuric acid and perchloric acid. Inorganic phosphorus present as phosphate forms a phosphomolybdate complex with molybdic acid. The complex is reduced by stannous chloride to a blue color which can be measured colorimetrically.

## SAMPLES :

Serum, Heparinized plasma or EDTA plasma. Serum can be stored for up to 7 days at +2 to +8°C and 2 days at +15 to +25°C.

## NORMAL VALUES IN SERUM:

	mg/dl	mmol/l
Phospholipid phosphorus	6 – 10	1.94 – 3.23
Phospholipids	150-250	15.52 – 25.84

## REAGENTS :

1 -	<b>Standard :</b> Phosphorus	4 mg/dl (1.29 mmol/L)
2 -	<b>Trichloroacetic acid</b>	0.6 mol/L
3 -	<b>Digestion Mixture:</b> Sulphuric Acid Perchloric Acid	2 mol/L 0.5 mol/L
4 -	Ammonium molybdate Sulphuric acid	0.32 mmol/L 0.5 N
5 -	<b>Reducing Reagent :</b> Stannous chloride <b>Dilute 50 times before use. (0.04 ml + 2 ml D. water)</b>	0.2 mol/L

## STABILITY :

The reagents are stable up to the expiry date specified when stored at +2 to +8 °C .

## DEPROTEINIZATION:

<b>Pipette into 10 ml centrifuge tubes</b>	
<b>Sample</b>	<b>0.020 ml</b>
<b>Trichloroacetic acid (R2)</b>	<b>1.0 ml</b>

Mix, and incubate for 5 mins at +20 to +25°C. Centrifuge for 10 min at 3000 rpm. Use the precipitate and discard the supernate. Stand the tube inverted on filter paper for few minutes.

## PROCEDURE :

**Dilute reagent 5 (0.04 ml + 2.0 ml dist water) .** Pipette into test tubes (phosphorus – free) for blank, standard and sample (the precipitate in centrifuge tube) :

	Blank (ml)	Standard (ml)	Sample (ml)
Standard (R1)	-	0.020	-
Sample	-	-	Precipitate
Digestion Mixture (R3)	0.5	0.5	0.5

Mix, heat gently in fume cupboard (use indirect flame) until the liquid becomes colorless and no fumes coming out. Let stand for 5 min. at this temperature, Cool then add:

<b>Reagent 4</b>	2.0	2.0	2.0
Mix, then add:			
<b>Reagent 5 (diluted)</b>	0.1	0.1	0.1

Mix. let stand for 10 min. at room temp. or 5 min. at 37°C . Read the absorbances of sample ( $A_{\text{Sample}}$ ) and standard ( $A_{\text{Standard}}$ ) against the blank at 650 nm. (620 – 700 nm.) . Color stable for few hours . Linearity up to 50 mg / dL (16.1 mmol/L) .

## CALCULATION :

The concentration of phospholipid phosphorus in the sample:

$$(\text{mg/dl}) = \frac{A_{\text{Sample}}}{A_{\text{Standard}}} \times 4$$

$$(\text{mmol/L}) = \frac{A_{\text{Sample}}}{A_{\text{Standard}}} \times 1.29$$

The concentration of phospholipid in the sample :

$$(\text{mg/dl}) = \text{Phospholipid phosphorus} \times 25$$

$$(\text{mmol/L}) = \text{Phospholipid phosphorus} \times 8$$

## REFERENCE :

- Zilversmit, D.B. and Davies, A.K. (1950), J.Lab. Clin. Med., 35, 155.
- Connerty, H.V., Briggs, A.R. and Eaton, E.H.Jr. (1961) Clin. Chem. , 7, 37, 580.

## QUALITY CONTROL :

For accuracy and reproducibility control:- Assayed Multi – Sera, Normal and Elevated

## PHOSPHOLIPIDS

**Colorimetric Method**  
**+2 to +8°C**                      **50 Tests**  
In vitro diagnostic use

**CAT. No.**                              **PL 17 11**

## REAGENTS

<b>R1 Standard</b>	<b>2</b>	<b>ml</b>
<b>R2 Trichloroacetic acid</b>	<b>50</b>	<b>ml</b>
<b>R3 Digestion Mixture</b>	<b>25</b>	<b>ml</b>
<b>R4 Color Reagent</b>	<b>100</b>	<b>ml</b>
<b>R5 Reducing Reagent</b>	<b>2</b>	<b>ml</b>

## CONTACTS

Tele: 02-33385184

Mobil: 019 – 349 20 77

Fax : 02-33385184 (102)

e.maile : [info@bio-diagnostic.com](mailto:info@bio-diagnostic.com)

Website: [www.bio-diagnostic.com](http://www.bio-diagnostic.com)

Adress: 29 Tahreer St., Dokki, Giza, Egypt