



**Introduction:** This can be used for determining the phospholipids content. Be careful when adding acid to the tubes. Most work should be done in the hood. It is best to practice using old samples and a standard curve before using important experimental samples.

**Protocol: Clin Chem Acta 121, 111-116. 1982**

- 1 - Prepare a standard curve phosphate curve in triplicate.  
(0, 25, 50, 75, 100, 150, 200, 400  $\mu$ l of 1 mM  $\text{KH}_2\text{PO}_4$ ) - calculate how many  $\mu$ g of phosphate are in each tube you will need this later.
- 2 - Add 25,  $\mu$ l of sample in separate tubes. Do in triplicate.
- 3 - Dry standards in heating block inside hood  
- dry organic solvent (lipid samples) under nitrogen.
- 4 - Add 100  $\mu$ l concentrated  $\text{H}_2\text{SO}_4$  to all tubes.
- 5 - Vortex and put tubes in the heating block for 10 min.
- 6 - Allow tubes to cool to room temperature and add 50  $\mu$ l of 6% hydrogen peroxide
- 7 - Vortex tubes and place in heating block for 40 minutes.
- 8 - Allow tubes to cool and add 2 ml of  $\text{H}_2\text{O}$ , mix well.
- 9 - Add 800  $\mu$ l of Color reagent to each tube.
- 10 - Boil samples on hot plate or heat block for 10 - 15 minutes or until highest standard turns a very dark blue.
- 11 - Transfer to 1 ml cuvettes and read absorbance at 797 nm
- 12 - Plot standards as absorbance vs.  $\mu$ g phosphate

**Solutions**

50 1 mM  $\text{KH}_2\text{PO}_4$

Color reagent

1:1 ammonium anhydride molybdic acid (0.625g/50ml): ascorbic acid (0.45 g / 50 ml)

Mix just prior to use.

6%  $\text{H}_2\text{O}_2$  - (1 ml of 30%  $\text{H}_2\text{O}_2$  and 4 ml of  $\text{H}_2\text{O}$ ) Make just prior to use