



## Phospholipid Phosphate Assay Protocol



MSUM Biochemistry

Itaya and Ui (1966) Clin. Chem. Acta. 14, 361-366

### Preparation of Reagent:

For 300 ml reagent combine:  
350 mg malachite green (Aldrich 21,302-0).  
3.2g Ammonium Molybdate.  
45 ml conc. HCl.  
255 ml H<sub>2</sub>O

Stir vigorously for 1 hr at room temperature.  
Filter through Whatman #1 paper.  
Store in stoppered bottle, reagent is good for one month.

### Assay:

Add small (10-50 $\mu$ l) samples of purified phosphoinositides to 13 x 100 borosilicate glass tubes. Evaporate to dryness. Add 100 $\mu$ l of conc. Perchloric acid. Boil samples using a bunsen burner in fume hood. Point tubes away from you, use a shield and wear safety glasses.

Samples will turn brown then clear as the lipids are digested. The perchloric acid will condense on the sides of the tubes. Cool to room temperature and dilute to 1 ml with distilled water.

The standard curve (made using sodium phosphate) is linear in the range 0-20 nmol PO<sub>4</sub><sup>2-</sup>. The slope will be 0.02-0.04 Au/nmol. Sample volume 200  $\mu$ l, add 2 ml reagent, mix, color develops in 5 min. Read OD at 660 nm. Samples are stable for 30 min before precipitate forms.

Calculation of phosphoinositide mass. Don't forget that PtdIns4P contains 2 moles of phosphate/mole lipid while PtdIns4,5P2 contains 3 moles phosphate/mole lipid.

### Molecular Weights:

PtdIns4P (diammonium salt) 1001.2  
PtdIns(4,5)P2 (triammonium salt) 1098.2