

Orthophosphate Plate Reader Protocol - AA

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Reagents

- Reagent 1 0.032 M Ammonium Molybdate (1235.86 g/mol)
Reagent 2 4.9 N Sulfuric Acid (concentrated is 36.76 N)
 *note: when using 1 M HCl to digest samples, use 1.54 N sulfuric acid instead of 4.9
Reagent 3 0.1 M Ascorbic Acid made fresh daily (176.12 g/mole)
Reagent 4 0.0045 M Antimony Potassium Tartrate (667.88 g/mole)

How to Make Reagents

Reagent 1:

- Dissolve 1.977 g Ammonium Molybdate in 50 mL of DI water

Reagent 2:

- Put approximately 30 mL of DI water in a 50 mL volumetric flask and slowly add 6.66 mL of concentrated Sulfuric Acid. The solution will heat up; once it has cooled to room temperature add DI water to bring the solution to 50 mL

Reagent 3:

- Dissolve 0.088 g of Ascorbic Acid in 50 mL of DI water

Reagent 4:

- Add 0.15g of Antimony Potassium Tartrate to 50 mL of DI water

Working Reagent: *made fresh daily by mixing reagents in this order (mixed after every addition)*

- 1.5 mL Reagent 1
- 5.0 mL Reagent 2
- 3.0 mL Reagent 3
- 0.5 mL Reagent 4

Loading 96 Well Plate

- Use a clean disposable clear polystyrene flat-bottom 96-well microplate
- Pipette the following into each cell:
 - 200 μ L of standard/sample
 - 50 μ L of Working Reagent; vortex @ 750 rpm for 30 minutes
- Following 30 minute reaction time, check absorbance @ 880 nm