

## **PHOSPHATE ASSAY**

(Chen *et al.* 1956)

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### **Reagents**

10% ascorbic acid:

10g ascorbic acid

water to 100ml

store at 4°C, good for one month

2.5% ammonium molybdate:

2.5g ammonium molybdate

water to 100ml

store at room temperature

6N sulfuric acid:

18ml concentrated acid

water to 108ml

store at room temperature

Reagent C, prepare fresh each day, keep at 4°C until use:

2 volumes water

1 volume 6N sulfuric acid

1 volume 2.5% ammonium molybdate

1 volume 10% ascorbic acid

### **Assay**

For each assay, add 1ml reagent C and 1ml sample to a cuvette. Cover with parafilm and invert several times to mix.

For your blank, mix 1ml reagent C and 1ml water.

Also include control samples with known molarities of phosphate ranging from 1µM to 200µM. These known samples will be used to create a standard curve that plots absorbance vs. molarity. This standard curve will be used to determine the molarities of your unknown samples.

Incubate samples for 1.5hrs @ 37°C.

For each sample, measure absorbance @ 820nm vs. your blank sample.

Determine the phosphate concentration in your unknown samples from your standard curve.

