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.