

## **Potato People:**

### **Fiske-Subbarow**

Read the Introductory material for the Fiske-Subbarow assay in the experimental protocol, i.e. make sure test tubes are well rinsed with dH<sub>2</sub>O before doing assay.

You need a good Pi Standard Curve (straight line) for the group to derive phosphate values for the remainder of the experiment.

### **Procedure for Hydrolysis:**

Using 0.1 mL of G-1-P solution, add 1.0 mL of 1N HCl.  
Place tubes in a boiling water bath in the hood for 7 minutes.  
Add 1.0 mL of 1N NaOH to neutralize (don't worry about measuring the pH).  
Adjust the volume to 3.0 mL by adding 0.9 mL of dH<sub>2</sub>O.

### **Preparation of Unhydrolyzed samples:**

Using 0.1 mL of G-1-P solution, adjust the volume of all tubes to 3.0 mL by adding 2.9 mL of dH<sub>2</sub>O.

### **General procedure used for all tubes (hydrolyzed and unhydrolyzed):**

Add IN ORDER:

- 1.0 mL Acid Molybdate Reagent
- 1.0 mL Reducing reagent
- 5.0 mL dH<sub>2</sub>O
- Allow color to develop for 15-20 minutes.
- Allow color to develop for 10 minutes in the heat blocks.
- Make sure spectrophotometer is set at 660nm.

### **Note:**

***Fiske-Subbarow without Hydrolysis is a measure of P<sub>i</sub>***

***Fiske-Subbarow after 7-Min Hydrolysis is a measure of G-1-P***