

### **Group I: Glucose-1-Phosphate, Purification**

1. Potato People pack their columns with Amberlite IR-45, anion exchange resin.
2. At this point you have removed excess free Pi and cations now their G-1-P will stick to the Amberlite allowing all other impurities (starch) to wash through, kind of the final purification. Wash the IR-45 resin with a lot of d-H<sub>2</sub>O, at least 250 mL, through the column checking the pH of the effluent (water) until it is 9.0 or lower.
3. Make sure the stopper at the bottom of the column is secure, label the column and parafilm the top.

### **Group II: Wheat Germ, Isolation**

1. Today you need to do both the Biuret and Phosphatase assay on all of the samples you have saved/stored. The Biuret assay will tell you how much total protein is in the solution but not how much enzyme or if the enzyme is active.
2. The acid phosphatase assay is performed using the artificial substrate, PNPP. If there is enough active enzyme the PNPP will be converted into PNP (yellow color that can be read using the specs) and Pi. Then using Beer's Law:  $ABS = [ ] \times l (1 \text{ cm}) \times \epsilon (18.8 \times 10^3 \text{ L mole}^{-1}\text{cm}^{-1})$  you can determine concentrations of wheat germ acid phosphatase.
3. The PNPP is expensive and should be stored frozen, removed from the freezer in the AM along with the other samples.
4. Use 37°C heat blocks rather than water baths to do the acid phosphatase assays.
5. See hand-out (link).