

## Biology 222L Reagents and Media

1. Review this list of reagents and perform any necessary **calculations**. You are responsible for knowing how to do all calculations, even for reagents your group does not actually make. Record these calculations in your laboratory notebook.
2. Work as a group of 2-4 students to make one of the following reagents as assigned by your instructor. You will be making this **reagent for the entire class** and ultimately everyone's experiments will depend on your accuracy. Be sure to ask your instructor if you have questions about making up your reagent. Record the protocol you used to make your reagent in your laboratory notebook. Be sure to clearly label your reagent with the reagent name and concentration, and with the date and your initials.
3. Work in pairs to make 500 ml of **glucose-minimal salts agar** as indicated in your Ames Test handout.

### **Vogel Bonner Medium E (50X)** (Ames test - this recipe is for 200 ml)

Warm 135 ml distilled water at 45 °C in a 500 ml beaker. Add the following salts in the order given below. Dissolve each salt completely before adding the next salt. Adjust the volume to 200 ml. Distribute into two bottles and sterilize at 121°C for 20 minutes with the caps loosened. Tighten the caps after sterilization when the solutions have cooled.

2 g magnesium sulfate (MgSO<sub>4</sub>)  
20 g citric acid monohydrate  
100g potassium phosphate, dibasic anhydrous  
35g sodium ammonium phosphate (NaH<sub>2</sub>NH<sub>4</sub>PO<sub>4</sub> - 4 H<sub>2</sub>O)

### **40% glucose solution** (Ames test - make 400 ml)

Sterilize this solution by filtration and distribute into 4 sterile 100ml bottles.

### **0.5 mM histidine/biotin solution** (Ames test - make 100 ml)

Dissolve the biotin by boiling the water. Place in a bottle and sterilize at 121°C for 20 minutes with the cap loosened. Tighten the cap after sterilization when the solution has cooled.

### **Homogenization buffer** (*Drosophila* experiment - make 20 ml)

10mM Tris-HCl (pH 8.2)  
1 mM EDTA  
25 mM NaCl  
200 ug/ml proteinase K

### **Luria Broth (LB)** (plasmid DNA experiment - make 200ml)

2 g NaCl  
2 g tryptone  
1 g yeast extract  
distilled deionized water to 200 ml

Place the media in a glass bottle and sterilize at 121°C for 20 minutes with the cap loosened. Tighten the cap after sterilization when the solution has cooled.