PROTOCOLS
Standard and Modified

Protocol ONE
Preparing Agar:

1. Turn on autoclave, if it beeps, boil up some water and pour it on the sensor to pre-heat the autoclave.

2. Wash flask(s) and graduated cylinder with water, then wash again with distilled water.

3. Check that electric meter is clean. Set electric meter to 0.

4. Measure with graduated cylinder 250mL of distilled water. Measure with electric scale 8.75g of LB media powder (for making agar). Do not fill graduated cylinder and flask(s) more than halfway.

5. Mix agar and water in flask(s). Put flasks into autoclave. Maximum capacity is 5 flasks. If instruments have lids, make sure to not screw the lid on tight.

6. Shut autoclave, set setting. Usually use “packs” for dry things and “liquid” for everything else.

7. Put tin foil wrapper on the top of flask(s), put a piece of TimeMed Time Tape to check temperature within autoclave.

8. Wait for autoclave to finish cycle, it usually takes 1 hr. When pressure is 0, open autoclave. Be careful of the hot steam coming out of the autoclave when door is first opened. Do not point the top of the flask(s) towards your face when taking them out of the autoclave.

9. Clean up. Unless toxic substances were measured, all garbage goes into normal trash.

Protocol TWO
Preparing Modified Petri Dishes:

Method ONE
Sterilize petri dishes with electrodes embedded.

1. Clean surface in sterile hood with 91% isopropyl alchohol. Use sterile prep napkins to wipe the surfaces dry.

2. Spray gloves with isopropyl alchohol.

3. Spray modified plates (with electrodes embedded) both inside and outside with isopropyl, then bring into hood.

4. Spray inside of plates with isolpropyl in hood, then leave in hood to air dry. Usually takes 20 - 25min.

5. Cover plates with lid, bring out of hood to pour agar.

Protocol TWO
Preparing Modified Petri Dishes:

Method TWO
Punch holes in sterilzed petri dishes and then embed sterile electrodes after agar solidifies.

1. Clean surface inside hood with 95% ethinal alchohol (another option besides isopropyl).

2. Clean soldering iron and stand with isopropyl, bring into sterile hood. Do not turn on soldering iron until it is completely dry because isopropyl and other sterilizing alchohols are high flammable.

3. Spray protoboard with isopropyl, wipe with sterile prep napkins and bring into hood. The protoboard will be used as a template for burning holes into the petri dish.

4. Burn holes into sterile petri dishes with soldering iron under hood. Do not open the lids of the petri dishes until they are in the hood.

5. Add alumminum tape to seal holes.

6. Put lid on petri dishes and bring out from under the hood to pour agar.

Protocol THREE
Grow Fresh Bacteria to Provide Material for Experiments:

1. Be sure to always grow fresh bacteria for your experiments. In this case, we are using the E. coli JM109 strain.

2. Take a sterile wooden stick and scrape some bacteria from a growing plate.

3. Streak the bacteria onto a fresh empty agar plate.

4. Put the newly streaked plate into the incubator to grow overnight.

5. E. coli's growth window is between 6 and 12 hrs. You will have bacteria by the next day.

Protocol FOUR
Using the spectrophotometer to count cells:

1. Turn on the spectrophotometer and wait 30min for it to heat up.

2. Scrape some JM109 cell from a prepared plate, delude it in LB liquid. In this case, we do not want to have too many cells and the mixture too dense. This is for better test results when monitoring bacterial growth rates. Take 500mL of this mixture and put it into a cubet.

3. Also prepare 500mL of LB liquid with no cells in it as a control.

4. Make sure the spectrophotometer is set to 600nm, this is the optimal obsorbance rate for cells.

5. Place the cubet containg the pure LB liquid into the spectrophotometer and set to 0.

6. Then put the cubet with the bacterial cells into the spectrophotometer and read the numbers. The max number is 1, which indicates 8 hundred million cells per mL, the minimum number should not be lower than 0.01. Based on the numbers, you can calculate the number of cells you spread onto an agar plate.