

## **Autoclaves in Room 3.7521**

### **IMPORTANT:**

Wear heat-resistant gloves when loading or unloading the autoclave.

Steam must be able to escape the containers. If liquid is autoclaved in capped bottles, do not fill the bottles more than two-thirds full. Open the lid slightly to release the pressure. Do NOT tightly close the lids. Preferably place the bottles into an autoclave proof tub with about an inch of water level in the tub.

### **Protocol for using the large autoclave**

To prepare 6 liters of autoclaved LB media for large-scale bacterial growth:

1. Fill the flasks with 1 Liter each of Nanopure water.
2. Weigh 25 g of the LB media mix for each of the 6 individual flasks and add them to the flasks. It is less messy to add the LB mix after the water.
3. Cover the flasks with two ample sheets of aluminum foil. The aluminum should reach far down the neck of the flask.
4. Paste an autoclave tape on one of the flasks, to confirm that the media was autoclaved properly (When the temperature and pressure is ideal for autoclaving, the color of the stripes in the autoclaving tape will change to black and that is an indication of a properly autoclaved media).
5. Sign –up the notebook for using the autoclave with an ‘L’ for ‘Large’, phone extension x6-3681, room number 3-8540, Kielkopf lab.
6. Check the autoclave is unpressurized and open the autoclave door. Use the union slide and black knob to engage (and later disengage) the hooks of the support cart in the two indentations at the bottom of the door.
7. Roll the autoclave racks out onto a support cart. Load the flasks. Roll the racks back in. Disengage the support cart. Close and lock the autoclave door.
8. “Cycle select”, “Liquid 30 cycle” and then “start cycle”.
9. It takes approximately 1.5 hours to complete the cycle. Allow an extra 10 minutes to cool down and then get the flasks out promptly to avoid “burnt” LB.
10. Take the flasks out using the support cart and close the door. Mark finished in logbook.
11. If using the media on the next day, store it in room temperature. Otherwise, if the LB is to be used later in the week, store it in the cold room. The flasks should be taken out of the cold room and kept at room temperature to warm up on the evening of inoculating growths for the large-scale culture.

## **Protocol for using the small autoclave**

To autoclave bottles of LB for small scale cultures or liquids (water, PBS) for tissue culture:

- Sign up for one of the small autoclaves in the notebook: S1 or S2, phone x6-3681 and room number 3-8540, Kielkopf lab.
- Ensure drain is closed, then fill pan to level indicated on autoclave from hoses in sink.
- Flip switch to slow (liquids), set time to 25-30min (depending on volume)
  - (for dry objects, e.g., spatulas or tips, flip switch to Fast, time 20-25min)
- Check autoclave is unpressurized and temperature has come down before opening the door to retrieve autoclaved materials.
- When finished, open drain and leave autoclave door open. Mark finished in logbook.