

Protocol for pouring LB plates

1. For 12 plates, make a solution of:

- 300 mL of DI H₂O
- 11.1 g of LB agar powder
 - To give a concentration of 37 g of LB per 1 L of DI H₂O

2. Microwave the LB media to dissolve the solution.

3. Once the solution is fully dissolved, place the cap on very loosely or cover flask with aluminum foil.

4. Place a piece of autoclave tape on the cap!

- This will show whether or not the autoclave has reached sufficient temperature.

5. Place the LB solution in an autoclave tray and put enough tap water in the tray so the entire bottom has a slight covering of water.

6. Take this tray to the autoclave on the 6th floor of Storer or the 3rd floor of Hutchinson and set the autoclave to the liquid setting for 30 minutes.

7. When the autoclave is finished, take the LB solution back to the lab and allow the LB solution to cool until it is bearable to the touch (around 55 °C).

8. Once it is cool enough, add your antibiotic and swirl.

9. Use a sterile 25 ml pipette to aliquot ~25 mls of the LB-antibiotic solution onto the plates.

- The amount for each doesn't have to be exact, just enough to cover the whole plate.

10. Cool plates and store them upside down in a plastic bag labeled with the antibiotic.

Adding antibiotic to LB plates

Make antibiotic stock solution (20 mg/ml):

- a. Weigh out 200 mg (0.200 g) of antibiotic. For spectinomycin, leave the glass jar at room temperature for at least 15 minutes before weighing the powder.
- b. Resuspend the powder in 10 ml of DI H₂O.
- c. Filter by running the solution through the disk-like filter attached to a plunger.
- d. Store at -20 °C.

Calculate the volume of antibiotic stock solution to add to plate:

$$C_1 * V_1 = C_2 * V_2$$

Example:

Ampicillin stock = 100 mg/ml

We want it to be 100 ug/ml in the plates.

$$100 \text{ mg/ml} * V_1 = 100 \text{ ug/ml} * 300 \text{ mls (e.g. how much LB you made)}$$

$$100 \text{ mg/ml} * V_1 = 0.100 \text{ mg/ml} * 300 \text{ mls}$$

$$30 \text{ mg} / 100 \text{ mg/ml} = 0.3 \text{ mls} = 300 \text{ ul}$$

Add 300 ul of the Ampicillin stock (100 mg/ml) for 12 plates.