

# Protocol for making Miller/Luria Broth (LB) Agar Plates

7/7/21

## Making LB agar:

For 20 petri dishes (100-90 mm diameter x 15 mm---per dish 15-20 mL of agar), make 500 mL of LB agar -you will have some leftover

### **To make 500 mL of LB agar (1.5% agar):**

5 g of tryptone (bacto-BD)  
2.5 g of yeast extract (bacto-BD)  
5 g of sodium chloride  
7.5 g of agar (bacto-BD)

### **for 250 mL**

2.5 g of tryptone (bacto-BD)  
1.25 g of yeast extract (bacto-BD)  
2.5 g of sodium chloride  
3.75 g of agar (bacto-BD)

1. Add dry ingredients to 500 mL of dH<sub>2</sub>O or diH<sub>2</sub>O in a 1 L glass bottle.
2. Mix well by either vortexing or shaking. Cover the opening of the bottle with its cap or aluminum foil (but do not make an air-tight seal!) and tape the bottle with autoclave tape. The autoclave tape will darken during the autoclave process if your sample has spent at least 10 min at 121 °C. Use lab tape to label the bottle with your initials, the date, and the bottle contents. The color of the prepared medium will be clear amber, slightly opalescent. The agar will not go into solution until it is heated in the autoclave.
3. Autoclave at 121°C for 15 minutes. Once the autoclave cycle is finished (0 PSI and temperature < 80°C), remove your LB agar using heat resistant gloves. If you store at room temperature (the mixture will solidify). To reheat the agar solution, loosen the cap and microwave in short bursts (30 sec). Wearing heat resistant gloves, swirl the solution in between bursts to help agar liquify.

### **If adding antibiotic to LB agar:**

4. Let the LB agar cool to ~50-55 °C. (Higher temperatures will degrade the antibiotics)
5. Add antibiotics and swirl to mix (do not shake, you don't want to add bubbles to the agar solution).
6. Pour plates immediately (time sensitive as the agar solution will be solidifying as it cools)

### Typical final antibiotic concentrations:

Kanamycin 50 µg/ml

Carbenicillin 100 µg/ml or Ampicillin 100 µg/ml  
Chloramphenicol 25 µg/ml  
Spectinomycin 50 µg/ml

\*stock solutions of antibiotics are stored at -20°C. Most stock solutions are diluted 1:1000 (1µL per 1mL of LB agar solution, e.g. 100 µL of antibiotic stock in 100 mL of LB broth)

**LB agar containing antibiotics cannot be stored. Reheating the agar medium will degrade the antibiotic.**

### Pouring agar plates:

While your samples are sterilizing in the autoclave, you should prepare your plate pouring station:

- To maintain sterility, you will work under the PCR hood
- Spray down the bench with a 70% ethanol solution and wipe down with a paper towel.
- Count out the appropriate number of plates and stack them on your lab bench.
- Label the bottom of the plates near the edge with your initials, the date and the medium they will contain including the identity of the antibiotic.

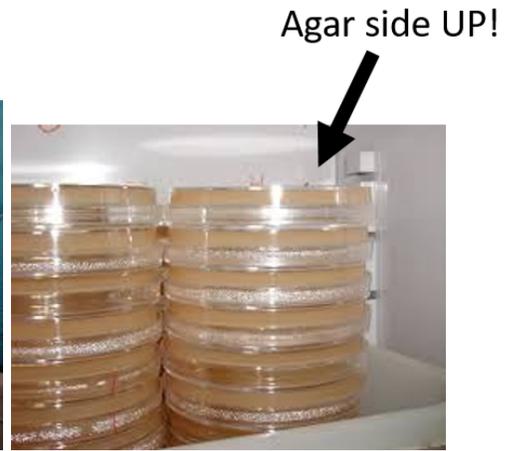
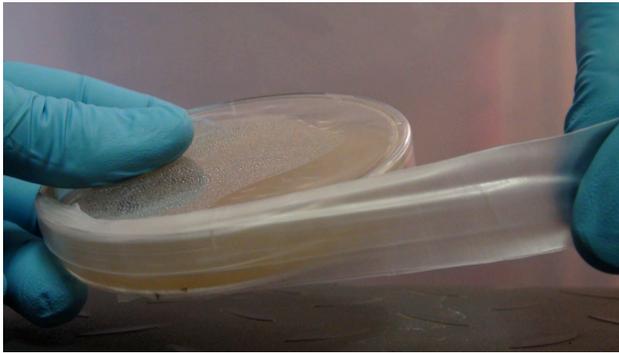
1. Cool the agar solution until it is just cool enough to handle, about 15-20 minutes. You should be able to hold your hand against the container reasonably comfortably for a few seconds. If the media is too cool, it will start to solidify in the container. If the agar solution starts to solidify, you can microwave it for 30 sec or until you don't see clumps of agar. If it is too hot, it will leave excess condensation on the lids.

2. Only pour 1 plate at a time. To prevent bubbles, gently pour agar into the petri dish. For the first dish, you can use a serological pipette to measure 10-20 mL of agar. Then use this plate as a guide to pour the remaining plates by eye. Swirl plate to ensure that the bottom of the dish is completely covered with agar. To prevent condensation, while pouring hot agar medium, stack agar filled petri plates on top of each other in sizes of 5-10.

3. Dry the plates in the PCR hood with the lid slightly off for 30 minutes (or in a 37°C incubator for 2–3 hours, or room temperature for 1-2 days. Drying the plate is very important for storing the plates and growing colonies on them.

**\*\*If you don't dry the plates, the moisture will evaporate and condense on the lid during storage or incubation and give you very wet plates which can drip onto the plates and spread bacteria.**

4. Once the plates have dried, seal them with parafilm. Next, return the plates to the plastic sleeve they came in or place them in a plastic bag. Label the bag with the media type, antibiotic (if included) and the date, and **store agar side up** at 4°C (refrigerator).



Agar plates will keep for 4-6 weeks at 4°C.