

# Lysogeny Broth (LB) Agar & Neurospora Agar Preparation Protocol (2017)

1. To make 200 mL of LB agar weigh out the following into an appropriate size media bottle:

- 2g NaCl
- 2g Tryptone
- 1g Yeast Extract
- 3g Agar
- and (dH<sub>2</sub>O) to 200mL

**LB Broth Only-** Add all of these components except the agar. Mix thoroughly and autoclave.

## **Neurospora Agar Recipe:**

To make 1 Liter autoclave the following:

- 1). 15 g agar
- 2). 15 g sucrose
- 3). 20 mL Vogel salts

The recipe makes 10 (11 cm) petri dishes. Adjust amounts accordingly to suit your needs. Please note that this recipe is for Lysogeny Broth (LB) agar. If making only the broth component, omit the agar.

2. Swirl to mix - the contents do not have to be completely in solution, but any powder left on the sides of the flask will caramelize on the glass during autoclaving.

3. Place the cap of the media bottle on top **loosely**, and put autoclave tape on lid. If media bottles are not available use Erlenmeyer flasks and cover the top of the flask with aluminum foil and label with autoclave tape.

4. Autoclave using Program 1 (by pressing #1). Program takes ~45 minutes.

5. After removing the solution from the autoclave, allow the agar solution to cool to 70°C (60°C if adding antibiotics). You may use the oven to ensure proper cooling without solidifying.

\*Optional Stopping Point: It is acceptable to close the lid tightly and store the labeled media bottle at 4° Celsius. When ready to use, the media can be heated using microwave or hot water bath.

6. Label the bottom of plates per the “Microbial Growth Media and Culturing Protocol”

7. When pouring plates, keep your bench area sterile by working near a flame or Bunsen burner.

8. Add the appropriate amount of desired antibiotic or other additives (if needed) to the solution at **60°C** and swirl to mix. Alternatively, you may use a sterile magnetic bar and stirring plate.

9. Pour LB agar until petri dishes are half way full.

10. If pouring slants, pour into sterile test tubes and tilt the rack holding the test tubes on a solid surface or a thick book, making sure the medium inside the tubes is at a slanted position. Allow the medium to cool and solidify at this angle, which increases the surface area of the agar.

11. Place the lids on the plates and allow them to cool for 30-60 minutes (until solidified) then invert the plates. Let sit for several more hours or overnight.

12. To store, put petri dishes in plastic bags or wrap with parafilm and store in 4° fridge.