

## LB Agar Plates

### Overview

This protocol covers the making of antibiotic agar plates with media. This protocol makes 500mL of LB plates, but can be scaled appropriately.<sup>i</sup>

### Materials

- Millipore Water
- Colored marker (Not expo)
- 1L glass flask
- Stir bar
- LB media components
  - o Either 12.5g of LB premix or 5g NaCl, 5g Tryptone and 2.5g Yeast Extract
- Appropriate Antibiotic Stock
- Sterile petri dishes
- Agar (powdered or granulated work equally well)
  - o Not agarose.

### Procedure

1. To a labeled 1L flask, add 12.5g of LB premix or LB media components.
2. Add 6g of agar to flask.
3. Fill flask with Millipore water to 450mL. Add stir bar, heat and stir until mostly dissolved. Adjust volume to 500mL
  - o Note: Bottle should be autoclaved fairly soon after making (a few hours)
4. Cover with foil and autoclave on liquid cycle.
  - o Note: Do not allow to sit for too long after cycle finishes, as it agar will eventually cool and harden, which will require re-autoclaving.
5. Allow solution to cool until warm, but touchable with gloves.<sup>ii</sup>
  - o Note, if working with two or more different types of plates, you may have to keep one warm on a hotplate
6. Add 500μL of the appropriate 1000x stock antibiotic.
  - o Adding antibiotics while temperature is >60C leads to degradation of antibiotics
7. Bring solution and sterile petri dishes to dead air box.
  - o **Note: Do not tear bag, bag is used for storing finished plates**

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<sup>i</sup> Note that this protocol can be adapted to other media simply by replacing the LB media components with the media components of your choice (ex. M9). Taking appropriate steps (i.e. not autoclaving glucose when making M9)

<sup>ii</sup> This is to prevent degradation of antibiotics. This temperature should be ~60C when touchable. This is especially important with ampicillin, carbenicillin and other beta-lactam antibiotics.

8. Wipe down hood and gloves with ethanol.
9. Place dishes face up with lids on in the hood.
10. For each plate: Remove lid, and slowly pour liquid. Cover the whole bottom of dish, but do not overfill (25-30mL). Swirl plate to remove bubbles. Put plate back down, and place lid so that it semi covers the plate (to prevent condensation during cooling).
  - **Note:** Work quickly, and keep on solution stirring on hotplate (though not too hot) when not being poured. Once chunks begin to form, stop pouring plates. Those are bad plates.
11. Once all plates have been made, allow them to solidify (if you need more space, begin stacking the most solid plates).
12. Once plates have solidified, take the appropriate colored marker and mark each plate.
  - Chlor: Black
  - Amp: Blue
  - Kan: Red
  - Green: Tet
13. Stack plates and place them inside the bag they came in. Label the bag with lab tape, include the date, antibiotic type, and your initials. Tape the bag closed using lab tape.
14. Store at 4C. Plates are stable for at least one month, and usually up to 3 months.<sup>iii</sup>

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<sup>iii</sup> The most common routes of decay are fungal growth or over-drying of plates. Ampicillin plates will begin to lose effectiveness more quickly than others.