

Preparation of LB broth (liquid) medium:

- □ In a 1L autoclave bottle (orange cap), add:
 - 25g LB broth powder
 - 1000mL MiliQ water
- Swirl to mix. Powder may not dissolve completely, that is ok but avoid clumps.
- Replace the cap to the bottle but leave it slightly loose for pressure equalization to occur. Place a fresh piece of autoclave tape on the top.
- Autoclave (121°C, 20 minutes) on LIQUID cycle, be sure to add water to the autoclave basin before starting the cycle! (this creates the necessary vapor pressure to prevent the liquid from evaporating in the autoclave)
- Cool to RT before use. Do not tighten cap until cool.

*****DO NOT add antibiotics before autoclaving!*****

Preparation of LB agar plates: (~30-35 plates)

- □ In a 1L autoclave bottle (orange cap), add:
 - 37g LB Agar powder
 - 1000mL MiliQ water
- Swirl to mix. Powder may not dissolve completely, that is ok but avoid clumps.
- Add a fresh piece of autoclave tape.
- Autoclave (121°C, 20 minutes) on LIQUID cycle – be sure you add water to the autoclave basin before starting the cycle!
- After cycle is complete, remove to benchtop to cool to ~50°C. (this is still quite warm, but cool enough to leave your gloved hand on the outside of the flask for several seconds without burning it. The media should still be liquid but should be just beginning to thicken). If you are not preparing your plates right away, keep the liquid in the oven (where we lyse mouse toes) for a few hours.
- If you are adding antibiotics, add them at this point (when the liquid is ~50°C warm), and swirl the flask vigorously for several seconds to mix. If you add antibiotics when the medium is too hot, the antibiotics may inactivate.
- Have Petri dished ready. Use Falcon, Cat. No. 351029.
- Pour out the media into petri dishes as follows: Remove the lid to a dish, pour just enough LB agar into the petri dish to completely cover the bottom of the dish, then replace the lid.
- Work quickly!! The agar will harden soon once it is cool enough to add antibiotics. You should finish pouring dishes within 5 minutes of adding the antibiotic.
- Helpful hint: to avoid condensation, pour plates in small stacks of 10 plates together. The only plates that will accumulate condensation on the lid are the ones on the top of the stack!
- Label the plates by drawing vertical lines (with marker) along the edges of the plates. Our code is:
 - Carbenicillin → 1 black line and 1 blue line
 - Kanamycin → 1 black line and 1 red line
- When the agar has hardened, store plates back in the plastic sleeve the petri dishes come in. Dishes should be stored UPSIDE DOWN in the cold room.

	Stock concentration	Working concentration (broth or agar)
Carbenicillin (a.k.a Carb)	100mg/mL	100µg/mL (dilute 1,000x)
Kanamycin (a.k.a. Kan)	50mg/mL	Typically 50µg/mL (dilute 1,000x)