

## Preparation of LB liquid medium:

- In a 1L autoclave bottle (orange cap), add:
  - 25g LB broth powder
  - 1000mL ultrapure water
- Swirl to mix. Powder will not dissolve completely, that is ok.
- 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 (This is program button 1 on the autoclave)– 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.

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

- Prepare 1L of LB liquid (25g broth powder per 1L) in a 2000mL Erlenmeyer flask.
- Add 15g (1.5%) bacteriological agar
- Swirl to mix. Powder will not dissolve completely, that is ok.
- Add a fresh piece of aluminum foil to cover the top, and add a fresh piece of autoclave tape.
- Autoclave (121 C, 15 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. (As a ballpark, 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 adding antibiotics, add them at this point, and swirl the flask vigorously for several seconds to mix.
- Set out your plates, and label the bottom with the appropriate antibiotic: A=ampicillin, C=chloramphenicol, K=kanamycin, T=tetracycline, S=streptomycin
- Pour out the media into petri dishes as follows: Remove the lid to a dish, and remove the foil cover to the flask. 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 4-5 plates, then stack up to 10 plates together. The only plates that will accumulate condensation on the lid are the ones on the top of the stack!
- Store plates back in the plastic sleeve the petri dishes come in (that means you need to take them out carefully so as not to damage the sleeve). Dishes should be stored UPSIDE DOWN in the cold room.