Preparation and Pouring of LB agar plates

LB (Luria-Bertani) agar plates are used in many molecular biology procedures. Each plate requires addition of ~20 mL LB agar. Many plates can be prepared in advance and stored in the refrigerator in room 125 (next to the fume hood). Commonly, 500 mL of LB agar is prepared (for ~25 plates). Keep in mind how many plates are needed before starting.

LB agar recipe (pH 7) – 1 L

Component	**Amount**	**Concentration in solution**
Tryptone	10 g	1.0%
Yeast extract	5 g	0.5%
NaCl	10 g	1.0%
Agar*	15 g	1.5%

* Agar is added *after* bringing the solution to final volume

In a 2 L Erlenmeyer flask, dissolve the above ingredients in 950 mL dH_2O. Adjust the pH of the solution to 7.0 (see Corning pH meter 140 protocol) with 1 M NaOH. Bring the volume up to 1 L with dH_2O. After adjusting volume, add 15 g agar before autoclaving. Wrap aluminum foil over the mouth of the flask, and autoclave on the liquid cycle (see autoclave procedure).

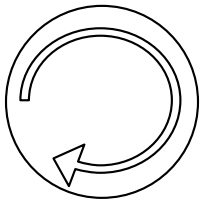
After autoclaving, move the flask to a 55 °C water bath. When the solution equalizes to the water bath, antibiotics may be added. For the TOPO TA cloning Kit (Invitrogen), 50 µg/mL ampicillin or 50 µg/mL kanamycin is recommended. Stocks of these antibiotics are usually prepared at a concentration of 100 mg/mL, divided into 100 µL aliquots, and stored at -20 °C. Plates must be poured inside of the Edge GARD laminar flow hood in room 125.

Pouring plates:

Important guidelines:

Pouring plates can be tricky procedure. Many find difficulty in pouring the right amount of LB-agar into each plate. To pour 20 mL consistently, pour gently until the bottom of the dish is completely covered.

1. Remove the desired number of dishes, and label the bottoms of all plates with the medium type (LB), type of antibiotic (if any), date, and initials of the preparer. Importantly, only mark the outside edges of the plates like below:



2. Assemble the labeled plates into stacks of five with the tops facing upwards. Remove the LB-agar solution from the 55 °C water bath, and place it inside of the hood.
   *The LB-agar solution will likely look heterogeneous, with a darker layer on the bottom of the flask and a lighter layer above it. The flask must be swirled gently to mix the lower agar-rich layer with the rest of the solution. Keep in mind that the agar will solidify in the flask if the plates are not poured immediately. The solidified agar cannot be autoclaved again because the reheating process breaks down nutrients in solution.
3. Placing your hand over the stack of plates, lift the lid of the bottom dish (and the four dishes on top of it). Gently pour ~20 mL of LB-agar into the bottom dish, taking care not to introduce bubbles to the solution. Replace the lid to the bottom. Continue pouring dishes in the same manner for the remaining plates. Between stacks, swirl the flask for a few seconds to keep the solution well mixed.
4. When finished pouring, do not disturb the stacks of plates. The LB-agar must fully solidify before the plates can be moved. When plates have completely set, assemble plates into stacks of 25 facing up. Carefully slide the plastic sleeve the dishes came in over the top of the stack. When the plates are all inside of the sleeve, slowly invert the sleeve. All plates will now be upside-down. Plates should be stored inverted to prevent condensation from resting on the agar growth surface. Seal the opening of the bag with a piece of pressure sensitive tape, and label the tape with the type of medium and any antibiotics added. Place the sleeve into the refrigerator next to the fume hood in room 125. Do not place the plates adjacent to the back wall of the fridge; sections of agar may freeze.