Making LB Agar Plates

Batch makes about 40 plates.

Making the LB Agar

1. Add 250 mL of dH2O to a graduated cyclindar.
2. Weigh out 20g of premix LB Agar powder (VWR DF0445-17) or:
3. 5.0 g tryptone
4. 2.5 g yeast extract
5. 5.0 g NaCl
6. 7.5 g agar
7. Mix powder well to bring into solution
8. Add dH2O to total volume of 500 mL and transfer to 1 L flask
9. Put on stirring hot plate and heat to boil for 1 min while stirring.
10. Transfer to 1 L pyrex jar and label with autoclave tape.
11. Autoclave at liquid setting for 20 minutes in a basin making sure to loosen top
12. Let agar cool to ~55C (you should be able to pick up the jar without a glove)

Pouring the Plates

1. Make sure bench top has wiped down with bleach/EtOH.
2. Remove sterile Petri dishes (VWR 25384-208) from plastic bag (save the bag for storage).
3. Pour a thin layer (5mm) of LB Agar (~10mL) into each plate being careful to not lift the cover off excessively (you should be able to just open up enough to pour).
4. Swirl plate in a circular motion to distribute agar on bottom completely.
5. Let each plate cool until its solid (~20 minutes) then flip so as to avoid condensation on the agar.
6. Store plates in plastic bags in fridge with: name, date and contents (note any additive).

Special Additives (to be added to LB Agar right before pouring plates)

Ampicillin (VWR 80055-786) 50 mg dissolved in a small amout of dH2O (concentration 100 ug/mL)

X-gal (VWR IB02260) 50 mg dissolved in a small amouth of DMSO

Stock solutions

Ampicillin 20mg/mL 200mg in 10mL dH2O (store at 4 in 1mL aliquots) use 50uL on each plate

IPTG (VWR EM-5800) 100mM 238 mg IPTG in 10mL dH2O (store at –20 in 1mL aliquots) use 40uL on each plate

X-gal 40 mg/mL 400 mg X-gal in 10mL DMSO (store at –20 in 1mL aliquots foil wrapped tubes) use 40uL on each plate