

Pouring Agar Plates

This recipe is for 500 mL of LB agar. This makes about 20 plates (1 bag).

5 g bacto tryptone
2.5 g yeast extract
5 g NaCl
7.5 g bacto agar

1. Add solids and 500 mL of deionized water to a large bottle or flask. Note the bacto agar will not dissolve until melted in the autoclave; but all other ingredients will dissolve.
2. Autoclave 20 minutes on LIQUID cycle.
3. Allow flask to cool on bench or in water bath.

Obviously, you don't want the solution to cool too much or it will start to solidify. You also don't want to add antibiotics when it is too hot since the heat will degrade the antibiotics. A good temperature to aim for is ~50-55 °C. At this temperature, the flask will still be pretty warm to the touch, but cool enough that you can place your hands on it and hold them there for several seconds.

4. Add antibiotics. Mix well by swirling.
Typical antibiotic final concentrations:
Kanamycin 30-50 µg/ml
Carbenicillin 50 µg/ml
Ampicillin 100 µg/ml

IMPORTANT NOTE: Ampicillin and carbenicillin stocks must be kept in the -80 C. They only last for 3-6 months if they are made from the sodium salt.

5. Pour plates.
It is best to do this next to a flame for reasons of sterility. This is especially important if your plates do not contain antibiotic.
Fill each plate 1/2 - 2/3 full.
6. Flame the air bubbles briefly to pop them if they formed. Solidified air bubbles are difficult to spread bacterial culture over.
7. Allow plates to cool at room temperature overnight. (To quickly cool plates, you can put them in the hood with their covers slightly open. This may cut dry time to a couple hours.)
8. When cool, put plates back into their sleeve, seal bag, LABEL (antibiotic and **DATE**), and store at 4 °C.