

Protocol for Pouring LB-Agar Plates by TCH 1/27/02

1. Set the water bath temperature at 55°C.
2. Use approx. **30% total power** of microwave oven to melt the LB-agar stock. You may have to run and stop the microwave every 2-3 min and shake the bottle. However, if you use freshly autoclaved LB-Agar, jump to the next step.
3. Cool down the melted LB-agar in the 55°C water bath.
4. After the bottle cools down to about 55°C, add appropriate amount of antibiotics (ie., Amp, Kan, or Strep, all stocks=1000x) and shake well (**Note**: It is important to add the antibiotics **ONLY after the agar is cooled down to about 55°C**, or the antibiotics may be inactivated).
5. Remove petri dishes out of the bags (but keep the bags). Mark the side of petri dishes: **Red line for Amp; Blue line for Kan.**
6. Turn on a flamer attached with a long pipe/tube.
7. Pour approx. 20-30ml of Agar/antibiotics mix to each plate, and pour 5-8 dishes at once. Flame the surface of dishes immediately to remove any air bubbles.
8. Allow agar to solidify (approx. 10-30min). Invert the plated upside down and leave at room temperature for overnight. Stack them back to the bags, date and keep at 4°C.

NOTE: Usually 700ml LB-Agar makes about 40 plates.