SUPPLY LIST

Media and chemicals: tryptone, yeast extract, bacterial agar, NaCl, 1 N NaOH, ampicillin, kanamycin

BACTERIAL MEDIA

Lural Broth (LB), 1 liter

- tryptone 10 g
- yeast extract 5 g
- NaCl 5 g
- 10 N NaOH 100 µl

Mix until ingredients are dissolved.

*Plates require 1.5% agar (see step three below)

1. Large cultures: place in appropriate sized Erlenmeyer flask, cover opening with aluminum foil (Never fill flask more than 50% full, i.e. 500 mls in a 1 liter E. flask)
2. Tube media: Dispense into autoclavable tubes and loosely cap. (Never fill more than 50% full.)
3. Plates: Add 1.5 % w/v bacterial grade agar. (Routinely place 500 mls LB in 1 liter E. flask, add 7.5 g agar, cover flask with foil and autoclave)

   Autoclave media: 121.0 ºC, 15 psi, 25 minutes
   Cool media until you can hold flask - but not below 50ºC. Aseptically pour into plates at 20 - 25 mls per plate. 500 mls media should make 20-25 plates.

If required, aseptically add antibiotics before pouring but after media has cooled to ~ 50ºC.

Antibiotic stocks
  - ampicillin (10 mg/ml) - final concentration 100 ug/ml (5 mls stock/500 mls media)
  Mode of action: Bacteriocidal; only kills growing E. coli; inhibits cell wall synthesis by inhibiting formation of the peptidoglycan cross-link.
  Mode of resistance: β - lactamases hydrolyzes ampicillin before it enters cell. (1.5 mls/500 mls media)

  - kanamycin (10 mg/ml) - final concentration 30 ug/ml )
  Mode of action: Bacteriocidal; inhibits protein synthesis; inhibits translocation and elicits miscoding
  Mode of resistance: Aminoglycoside phosphotransferase inactivates kanamycin

Store all plates inverted, in bags at 4ºC.

Label bags: (when using antibiotics, label individual plates with an A or K depending on the antibiotic they contain)

- Media
- Date of preparation
- Last name of preparer.