

## Liquid mediums and Agarose and Plates

### LB agar for 1L (2x YT)

- Tryptone 10g (16g)
  - NaCl 10g (5g)
  - Yeast Extract 5g (10g)
  - Agar 15g
1. Weight all the things above
  2. Mix Tryptone, NaCl, and Yeast Extract and put it in beaker
  3. Add around 700-800 ml ddH<sub>2</sub>O into the beaker
  4. Put stir bar inside the beaker and stir it until it dissolved
  5. Put Agar into 1L bottle and pour the mixture into the bottle
  6. Top up the volume until 1L with ddH<sub>2</sub>O
- \*Leave out agar if making liquid LB

### LB plates plus antibiotics

- After autoclaved, when the agar is not too hot to touch, add antibiotics
1. Ampicillin – add 1 ml ampicillin stock (100mg/ml) per liter of agar so the final concentration is 100µg/ml. Mark the plate with “A” on the side.
  2. Kanamycin – add 1 ml kanamycin stock (50mg/ml) per liter of agar so the final concentration is 50µg/ml. Mark the plate with “K” on the side.
  3. Chloramphenicol – add 1 ml chloramphenicol stock (25mg/ml) per liter of agar so the final concentration is 25µg/ml. Mark the plate with “CAM” on the side.

### SOB

Deionized H<sub>2</sub>O, to 950 ml  
Tryptone, 20 g  
Yeast extract, 5 g  
NaCl, 0.5 g

For solid medium, please see Media Containing Agar or Agarose.

Shake until the solutes have dissolved. Add 10 ml of a 250 mM solution of KCl. (This solution is made by dissolving 1.86 g of KCl in 100 ml of deionized H<sub>2</sub>O.) Adjust the pH of the medium to 7.0 with 5 N NaOH (approx. 0.2 ml). Adjust the volume of the solution to 1 liter with deionized H<sub>2</sub>O. Sterilize by autoclaving for 20 minutes at 15 psi (1.05 kg/cm<sup>2</sup>) on liquid cycle. Just before use, add 5 ml of a sterile solution of 2 M MgCl<sub>2</sub>. (This solution is made by dissolving 19 g of MgCl<sub>2</sub> in 90 ml of deionized H<sub>2</sub>O. Adjust the volume of the solution to 100 ml with deionized H<sub>2</sub>O and sterilize by autoclaving for 20 minutes at 15 psi [1.05 kg/cm<sup>2</sup>] on liquid cycle.)

### SOC

Deionized H<sub>2</sub>O, to 950 ml  
Tryptone, 20 g

Yeast extract, 5 g  
NaCl, 0.5 g

For solid medium, please see Media Containing Agar or Agarose.  
SOC medium is identical to SOB medium, except that it contains 20 mM glucose. After the SOB medium has been autoclaved, allow it to cool to 60°C or less. Add 20 ml of a sterile 1 M solution of glucose. (This solution is made by dissolving 18 g of glucose in 90 ml of deionized H<sub>2</sub>O. After the sugar has dissolved, adjust the volume of the solution to 100 ml with deionized H<sub>2</sub>O and sterilize by passing it through a 0.22-µm filter.)

### Terrific Broth (TB)

TB is a highly enriched medium used for the cultivation of bacteria.

900 ml TB medium Tryptone 1.2%  
100 ml 10X TB salts phosphate buffer 89 mM

TB medium  
1.2% tryptone 12 g/L  
2.4% yeast extract 24 g/L  
0.5% glycerol 5 g/L

Dissolve tryptone, yeast extract and glycerol in water to a final volume of 900 ml and autoclave for 15 min at 121°C. Let cool down to room temperature before adding the 10X TB

Salts 10X TB salts phosphate buffer 89 mM  
0.17 M KH<sub>2</sub>PO<sub>4</sub> 23.1 g/L  
0.72 M K<sub>2</sub>HPO<sub>4</sub> 125.4 g/L

Dissolve  
23.1 g of KH<sub>2</sub>PO<sub>4</sub> and 125.4 g of K<sub>2</sub>HPO<sub>4</sub>, dissolve in water to a final volume of 1 L and autoclave for 15 min at 121°C.

Finally combine the two components the medium and salts, be reminded to autoclave the salt and medium separately.