

PROTOCOL FOR BACTERIAL LB AGAR

LB

You need

1. Bactotryptone GibcoBRL #30392-021
Or DIFCO #0123-17-3 (=select peptone 140)
2. Yeast extract GibcoBRL #20047-056 or DIFCO #0127-01-7
3. Sodium chloride
4. 10 litre plastic beaker (not sterile)
5. Magnetic stirring bar
6. Access to autoclave
7. BactoAgar (BD 214010)

Method

- A. Place the beaker on the balance with the large stirring bar inside.
- B. Add the following quantities of solid.

Solid	Amount (for 5 litres)
Tryptone	50g
Yeast extract	25g
NaCl	50g

- C. Remove the beaker from the balance and add purified water to a total of 5 litres.
- D. Stir on the magnetic stirrer until the solids are well mixed (not everything will go into solution).
- E. Dispense into 1 litre Duran flasks and label with 'LB, the date, your initials' on a strip of autoclave tape on the bottle.
- F. Add 15g of BactoAgar into each of the 1 litre bottles.
- G. Screw the top on **loosely** (if it is too tight the bottle may explode in the autoclave). Tape the top on the bottle with autoclave tape.

H. Autoclave. Once the bottles have cooled, remove from autoclave, remove tape from tops, screw down the tops and place in the cupboard.