

# TARDIGARD

p r o t o c o l

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Preparation of culture media, agar plates, antibiotics and general necessities.  
LB broth and LB agar for culturing bacteria, agar plates to grow clone and antibiotics for selection.

## 1. LB agar and LB broth(liquid)

### 1.1 Materials

1L medium, each need to be divided by 5 if you are making 200ml medium

NaCl

Peptone

Yeast extract

Agarose

### 1.2 ingredients

NaCl 10g

Peptone 10g

Yeast extract 5g

Agarose 15g (only add if you need to make LB agar) (do not add when making liquid medium)

### 1.3 method

use ddH<sub>2</sub>O to wash the bottle

add few ddH<sub>2</sub>O (prevent powdered ingredients stick on the wall of container)

weigh and add all ingredients

add water to 200ml mark

shake thoroughly and let it mix

then go autoclaving, see part 2

## 2. Autoclaving

check if the bucket with condensate water is full

if so, empty it

make sure your bottle is half opened (prevent cracking as well as let the asepsis steam enter)

close and lock the lid

start the programme

## 3. Antibiotic stocks

### a) Ampicillin

- Dissolve 0.25 g ampicillin in 5 ml MiliQ.
- Mix/vortex so that all the ampicillin goes into solution.
- Filter into a falcon tube using a syringe and a 0.22 µm filter for sterilization.
- Aliquot into smaller Eppendorf tubes.
- Store at -20 °C under dark conditions.

### b) Chloramphenicol

- Dissolve 0.136 g of chloramphenicol into 4 ml 100% ethanol.
- Mix/vortex so that all the chloramphenicol goes into solution.
- Filter into a falcon tube using a syringe and a 0.22 µm filter for sterilization.
- Aliquot into smaller Eppendorf tubes.
- Store at -20 °C.

4. Pouring media into plates

Heat LB agar in microwave oven

Make sure the lid is half open

Allow it to cool down to 60 degree Celsius in water bath

Add antibiotic in 1:1000 ratio

Mix thoroughly and add approximately 10 ml to each plate

Left the lid half open and wait it to dry

Store it inside clean plastic bag inside fridge

Pre-heat and pre-dry it in drying oven or incubator before use next time