

Preparation of Bacterial Growth Broths and Agar, Antibiotics and IPTG

Luria-Bertoni broth (LB)

Requires:

10 g tryptone

5 g yeast extract

5 g NaCl

1 ml of 1 M NaOH

Make to a final volume of 1 litre with distilled water. Autoclave.

LB-agar

Add agar to LB to a final concentration of 1.5% [m/v] and autoclave. When sending to the autoclave, write "**KEEP AT 60°C**" on the bottle so that the person in charge of operating the autoclave puts the autoclaved LB-agar in the 60°C oven in the autoclave room on the 2nd floor of MCB.

Kanamycin sulfate (working concentration = 25 µg/ml)

Stock solution is 10 **mg/ml** in distilled water. This is filter-sterilised through a 0.22 µm filter and 500 µl aliquots are stored at -20°C until required. The final working concentration of

kanamycin is 25 µg/ml. Take note that our own batch of **kanamycin** can be kept at room temperature and does not require refrigeration like other antibiotics. Also, check the bottle label to see how pure the **kanamycin** is. It may only be around 76% pure, in which case, you would have to weigh out 100/76 times more **kanamycin** than if one had a 100% powder.

Chloramphenicol (working concentration = 34 µg/ml)

Stock solution is 34 **mg/ml** in 100% ethanol. This does not require filter-sterilisation. 500 µl aliquots are stored at -20°C until required. The final working concentration of chloramphenicol is 34 µg/ml, i.e. a 1/1000 dilution of the stock. This antibiotic is part of the pool on the second floor and you can get it in the walk-in fridge right next to the balance room.

Ampicillin, sodium salt (working concentration = 50 µg/ml)

Stock solution is 25 **mg/ml** in distilled water. This is filter-sterilised through a 0.22 µm filter and 500 µl aliquots are stored at -20°C until required. The final working concentration of ampicillin is 50 µg/ml.

LB-agar plates

Pour molten LB-agar (direct from the 60°C oven) into sterile Petrie dishes (~30 ml per dish) and allow to solidify at room temperature. After the agar has solidified, transfer the plates into a 50°C oven and incubate, inverted, overnight to completely dry the agar. After this, seal the plates with strips of Parafilm and store inverted at room temperature until required.

LB-agar plates with antibiotics

Kanamycin & chloramphenicol or Ampicillin & chloramphenicol (where applicable): Mix 100 µl 10 **mg/ml kanamycin** with 20 µl 34 **mg/ml** chloramphenicol. Dip a glass spreader in ethanol and flame. Dispense the 120 µl of antibiotic mix onto the agar and spread with the glass spreader until the solution has been absorbed by the agar. Ensure that you distribute the antibiotics evenly across the plate. Leave the plates to dry completely and then add bacteria by loop or pipette and plate or streak.

Chloramphenicol only: Mix 100 µl distilled water with 20 µl 34 **mg/ml** chloramphenicol and spread as above.

SOB broth for electroporation

Requires:

20 g tryptone

5 g yeast extract

0.5 g NaCl

500 μ l of 1M NaOH

Make to a final volume of 1 litre with distilled water. Autoclave.

IPTG (isopropyl- β -D-thiogalactopyranoside)

Stock solution is 500 mM in distilled water and 1 ml aliquots are kept at -20°C . Molecular weight of IPTG = 238.3 g/mole. The cheapest and quickest supplier for IPTG is Roche. The final working concentration of IPTG is 1 mM.