Sugden Lab.

Common reagents for Bacteria prep.

Medium

LB medium

Bacto Tryptone	10 g
Bacto Yeast Extract	5 g
NaCl	10 g
distilled water	to a final volume of 1 L

1. Add tryptone, yeast extract and NaCl to 900 ml of distilled water.
2. Stir to dissolve, adjust pH to 7.0 with 5N NaOH.
3. Adjust the volume to 1 L with distilled water and autoclave.

2 x LB medium

Bacto Tryptone	20 g
Bacto Yeast Extract	10 g
NaCl	20 g
distilled water	to a final volume of 1 L

1. Add tryptone, yeast extract and NaCl to 900 ml of distilled water.
2. Stir to dissolve, adjust pH to 7.0 with 5N NaOH.
3. Adjust the volume to 1 L with distilled water and autoclave.

LBON medium (LB without NaCl)

Bacto Tryptone	10 g
Bacto Yeast Extract	5 g
distilled water	to a final volume of 1 L

1. Add tryptone and yeast extract to 900 ml of distilled water.
2. Stir to dissolve, adjust pH to 7.0 with 5N NaOH (~0.2 ml).
3. Adjust the volume to 1 L with distilled water and autoclave.
   -Note:For LBON plates, add 15 g bactoagar to each liter of medium prior to autoclaving.

YT medium

Bacto Tryptone	8 g
Bacto Yeast Extract	5 g
NaCl	5 g
distilled water	to a final volume of 1 L

1. Add tryptone, yeast extract and NaCl to 900 ml of distilled water.
2. Stir to dissolve, adjust pH to 7.0 with 5N NaOH.
3. Mess up to 1 L and autoclave.

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2 x YT medium

Bacto Tryptone	16 g
Bacto Yeast Extract	10 g
NaCl	5 g
distilled water	to a final volume of 1 L

1. Add tryptone, yeast extract and NaCl to 900 ml of distilled water.
2. Stir to dissolve, adjust pH to 7.0 with 5N NaOH.
3. Mess up to 1 L and autoclave.

SOB medium

Solution I

Bacto Tryptone	20 g
Bacto Yeast Extract	5 g
NaCl	0.5 g
1 M KCl	2.5 ml
distilled water	to a final volume of 1 L

Solution II

1 M $MgCl_2$	10 ml (Sterilize by autoclaving)
1 M $MgSO_4$	10 ml (Sterilize by autoclaving)

1. Add tryptone, yeast extract and NaCl to 980 ml of distilled water.
2. Stir to dissolve, add KCl, adjust pH to 7.0 with 5N NaOH, and autoclave and cool to r.t.
3. Just before use add Mg solution.

SOC medium

Solution I

Bacto Tryptone	2 g
Bacto Yeast Extract	0.5 g
1 M KCl	0.25 ml
1 M NaCl	1 ml
distilled water	to a final volume of 100 ml

Solution II

1 M $MgCl_2$	1 ml (Sterilize by autoclaving)
1 M $MgSO_4$	1 ml (Sterilize by autoclaving)

Solution III

2 M glucose	1 ml (Sterilize through a 0.22 μm filter unit.)

1. Add tryptone and yeast extract to 980 ml of distilled water.
2. Stir to dissolve, add NaCl and KCl, adjust pH to 7.0 with 5N NaOH, and autoclave and cool to r.t.
3. Add Mg solution and glucose solution.

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TB (Terrific Broth) medium

Solution I

Bacto Tryptone	12 g
Bacto Yeast Extract	24 g
Glycerol	4 ml
distilled water	to a final volume of 900 ml

Solution II

KH2PO4	2.3 g
K2HPO4	12.5 g
distilled water	to a final volume of 100 ml

1. Autoclave and cool solutions to r.t
2. Mix solutions under sterile conditions for a 1 L final volume.

Super broth

Bacto Tryptone	25 g
Bacto Yeast Extract	15 g
NaCl	5 g
distilled water	to a final volume of 1 L

1. Add tryptone, yeast extract and NaCl to 900 ml of distilled water.
2. Stir to dissolve, adjust pH to 7.5 with NaOH.
3. Mess up to 1 L and autoclave.

Plate Agar and Top agar

Plate agar: 12~15 g/L agar to LB or YT medium.
Top agar: 6~7 g/L agar to LB or YT medium.

-Note: Allow the medium to cool to 50C before adding antibiotics.

- **Storage Medium**

Note: Bacteria can be stored for up to 2 years in stab cultures or indefinitely in cultures contains glycerol.

Culture Containing Glycerol

1. To 0.85 ml of bacterial culture, add 0.15 ml of sterile glycerol (filter-sterilized) in a storage tube equipped with screw cap and vortex to ensure that the stock solution is evenly dispersed.
2. Freeze the culture at –70˚C for long-term storage.
3. To recover the bacteria scrap the frozen surface of the culture with a sterile inoculating needle onto the surface od an LB agar plate with the appropriate antibiotics and incubate the plate O/N at 37˚C.

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Stab culture

- Use the vials (2~3 ml) with screw-on cap fitted with rubber gaskets. Add molten LB agar until the vials are two third full. Autoclaved the partially filled vials (with cap loosely screwed on).
- Let it cool to r.t. and then tighten the cap. Store the vials at r.t. until needed.
- To store bacteria pick a single, well isolated colony with a sterile inoculating needle and stab the needle several times through the agar to the bottom of the viral. Replace and tighten the cap and store the viral in the dark at r.t.

Selection solutions

100 mM IPTG

1. Dissolve 23.8 mg IPTG/ml autoclaved deionized water.
2. Store in working aliquots at –20˚C.
3. Add 30 µl of IPTG for every 2.5 ml of top agar.
4. Alternatively spread 30 µl on 100 mm plates.

2% X-gal (or Bluo-gal)

The DMF and DMSP indicated for use in this protocol; are hazardous. Exercise care with these reagents.

1. Dissolve 20 mg X-gal (or Bluo-gal)/ml dimethyl formamide (DMF) or 50:50 DMSO:water, Store at –20 ˚C without freezing. (X-gal and Bluo-gal in 100% DMSO freezes). Protect from light.
2. Add 50 µg/ml of X-gal (or 300 µg/ml Bluo-gal) to cooled media and top agar. Alternatively 50 µl of a X-gal solution or 100 µl of Bluo-gal (20 mg/ml) can be spread on the surface of the plate.

Note: If the X-gal or Bluo-gal is spread on the plate, wait 30 min to 1 h before the plates are used so that it can soak into the plate.

Antibiotics solutions

Ampicillin (100 mg/ml)

1. Dissolve 100 mg ampicillin/ml autoclaved deionized water. Filter-sterilize through a 0.22 µm filter and store at –20˚C.
2. Add 1 ml ampicillin/L of medium for a final concentration of 100 µg/ml.

Chloramphenicol (3.75 mg/ml)

1. Dissolve 3.75 mg chloramphenicol/ml 95% ethanol and store at –20˚C.
2. Add 4 ml chloramphenicol/ L of medium for a final concentration of 15 µg/ml.

Kanamycin (50 mg/ml)

1. Dissolve 50 mg kanamycin/ml autoclaved deionized water. Filter-sterilize through a 0.22 μm filter and store at –20˚C.
2. Add 0.8 to 4 ml chloramphenicol/ L of medium for a final concentration of 10 to 50 μg/ml.

Streptomycin (25 mg/ml)

1. Dissolve 25 mg streptomycin/ml autoclaved deionized water. Filter-sterilize through a 0.22 μm filter and store at –20˚C.
2. Add 4 ml streptomycin/ L of medium for a final concentration of 100 μg/ml.

Tetracycline (3.13 mg/ml)

1. Dissolve 3.13 mg tetracycline/ml autoclaved deionized water. Filter-sterilize through a 0.22 μm filter and store at –20˚C.
2. Add 4 ml streptomycin/ L of medium for a final concentration of 12.5 μg/ml.

-Note: Tetracycline is light sensitive. Protect from light.
-Note: Prefilter the filter-sterilizing units with distilled water before use to remove any toxic material.