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Preparing LB Broth or Agar +/- Antibiotic

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In order for bacteria to be successfully cultured, they must be grown in the appropriate media. LB, also known as Lysogeny broth (also known as **Luria broth**, **Lennox broth**, or **Luria-Bertani medium**.), is a nutrient rich broth that is a standard for culturing *Escherichia coli*, as it allows for quick growth and high yields. Furthermore, addition of agar to LB broth creates a gel for bacteria to grow upon, and is therefore used for plating bacterial cultures on petri dishes for single colony isolation.

This procedure is to be used for the preparation of LB media and LB plates +/- antibiotic.

The formulations generally differ in the amount of sodium chloride, thus providing selection of the appropriate osmotic conditions for the particular bacterial strain and desired culture conditions. The low salt formulations, Lennox and Luria, are ideal for cultures requiring salt-sensitive antibiotics.

LB-Miller (10 g/L NaCl)

LB-Lennox (5 g/L NaCl)

LB-Luria (0.5 g/L NaCl)

This SOP includes instructions to make either 1litre or 120ml LB broth.

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<https://protocols.io/view/preparing-lb-broth-or-agar-antibiotic-b85qry5w>



LB Broth, LB Agar

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Chemicals

- Yeast Extract
- Tryptone
- NaCl
- Agar
- Antibiotic stock solution (as required)

Equipment

- Beaker
- Measuring cylinder
- Electronic balance
- Weighing boat
- Spatula
- Aluminum foil
- Distilled water
- Clean Erlenmeyer Flasks (50ml and 250ml)
- Sterile Petri dishes

The process is generally safe but safety lab procedure must be followed and avoid dust production and inhalation from the media.

Ensure you check that all the materials and reagents required for this process are available and assembled.

Weighing components for LB Broth

- 1 Weight all powders **Yeast Extract CAS 8013-01-2, NaCl CAS 7647-14-5 and Tryptone CAS 91079-40-2** as indicated in the table below using weighing boats and place powders into an appropriate size beaker, add the corresponding amount of dH₂O and mix using a magnetic stirrer.

LB media composition Reagent	Amount for 1L (g)	Amount for 120ml (g)
Yeast Extract	5	0.6
NaCl	5	0.6
Tryptone	10	1.2

- 2 Dispense the resulting 120ml mixture into the following flasks;
2x 50ml LB broth in 250ml conical flasks
1x 5 ml and 1 x 10 ml in 50 ml conical flask
- 3 Seal flasks with aluminum foil and autoclave to make the culture media sterile
- 4 Remove flasks from autoclave, label with production date and store at room temperature or +4°C until required.

Add appropriate antibiotic as required prior to use

Weighing components for LB Agar Plates

- 5 Weight all powders in a beaker as indicated in the table below, add the corresponding amount of dH₂O and mix using a magnetic stirrer (all will dissolve apart from agar).

LB media composition Reagent	Amount for 1L (g)	Amount for 120ml (g)
Yeast Extract	5	0.6
NaCl	5	0.6
Tryptone	10	1.2
Agar	15	2.4

- 6 Complete steps 2 and 3 above.
- 7 Remove the flasks from the autoclave and allow the media to cool to about 55°C (until they can be held by hand)
Add appropriate antibiotic, if required, prior to use

Adding Antibiotic

8 The case of Kanamycin 50µg/ml, Kanamycin Sulphate powder CAS 25389-94-0

50µg/ml final concentration Kanamycin (add 0.5µl per ml LB agar from a 100mg/ml stock solution prepared using this [protocol](#)).

Aliquoting LB Agar into Petri dishes

- 9 Promptly and smoothly pour LB Agar medium +/- antibiotic (approx. 15-20ml) into the base of Petri dishes, cover with Petri lid and allow to cool and completely solidify.
- 10 Label Petri dishes with manufacture date and antibiotic and store at +4°C until they are used if not used immediately.

To avoid contamination, plates should be discarded if not used after 1 month.