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Making LB agar plates

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Protocol status: Working

We use this protocol and it's working



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Abstract

Luria broth (LB) is a nutrient-rich media commonly used to culture bacteria in the lab. The addition of agar to LB results in the formation of a gel that bacteria can grow on, as they are unable to digest the agar but can gather nutrition from the LB within. When required the addition of an antibiotic to this gel allows for the selection of only those bacteria with resistance to that antibiotic - usually conferred by a plasmid carrying the antibiotic resistance gene.

See the link for more details: <https://www.addgene.org/protocols/pouring-lb-agar-plates/>

- 1 Purchase LB Agar from the media kitchen and autoclave it to melt

Content of LB Agar:

37 g pre-mixed powder consisting of:

- 5 g peptone
- 10 g peptone from casein
- 10 g sodium chloride
- 10 g g agar-agar

1 L Sterile H₂O

- 2 If the bacteria have a specific antibiotic resistance then add 1000x of that antibiotic to the melted LB agar before pouring, e.g. if we use ampicillin we add 100mg/ml to the LB in a way that the final concentration becomes 100ug/ml. So, for 200ml LB agar we add 200ul of 100mg/ul of stock ampicillin

	Antibiotic	Recommended Stock Concentration	Recommended Working Concentration
	Ampicillin	100 mg/mL	100 µg/mL
	Bleocin	5 mg/mL	5 µg/mL
	Carbenicillin	100 mg/mL	100 µg/mL
	Chloramphenicol	25 mg/mL (dissolve in EtOH)	25 µg/mL
	Cou	25 mg/	25 µg/



	mer myci n	mL(diss olve in DMS O)	mL
	Gent amy cin	10 mg/ mL	10 µg/ mL
	Kan amy cin	50 mg/ mL	50 µg/ mL
	Spe ctino myci n	50 mg/ mL	50 µg/ mL
	Tetr acyc line	10 mg/ mL	10 µg/ mL

Antibiotic Concentrations

- 3 Maintaining sterile techniques, pour the molten agar on large petri dishes (90mm) roughly about 30-45ml (don't need to be exact on the volume) and leave to dry overnight
- 4 Store the plates at 4C to be used later, ideally it should be used within 2 weeks but can be used for longer if not contaminated or dehydrated but never more than 3months