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Protocol 1: Making Agar Plates

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¹UCSC1 *Works for me*

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ABSTRACT

This protocol will assist you in making the agar plates for the CRISPR experiment. As you may have discovered in your prelab, the agar provides the bacteria with the nutrients needed to grow. Once your plates are made you may use an inoculation loop to streak them with bacteria. Over time your bacteria should grow and you will see little specs of colonies formed by your culturing. You will have to be very attentive to your plates for a successful experiment.

TimeLine

Prep:15

Heat agar and water: 5 minutes

Cool: 10 - 30 minutes

Plate: 5 minutes

repeat with LB strep/kan/arabinose agar: 30 minutes

Wait for agar to solidify: 1.5 hours

Flip: 1 minute

Hands on time: 1 hour

Wait time: 1 hour 40 minutes-2 hours

PROTOCOL CITATION

2020. Protocol 1: Making Agar Plates. **protocols.io**
<https://protocols.io/view/protocol-1-making-agar-plates-bnc9maz6>

FORK FROM

Forked from [Protocol 1: Making Agar Plates, Alyssa Ayala](#)

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GUIDELINES

You will be repeating this set twice: first with LB Agar, second with LB Strep/Kan/Arab Agar. The first set of plates will be for the initial bacterial culture. The second set of plates will have the antibiotics necessary for testing the success of the experiment.

MATERIALS TEXT

- 1 - scale
- 1 - apparatus with boiling water
- 4 - weigh paper sheets
- 1- spoon
- 1- heat resistant glove or cloth (to handle hot agar)
- 1 - erlynmeyer flask
- 1- piece of foil
- 1 - tube LB Agar *for **before** CRISPR*
- 1 - tube LB Strep/Kan/Arab Agar *for **after** CRISPR*
- 1 - Non-pathogenic E. coli bacteria Freeze Dried Tube (DH5α)
- 1 - Sterile Water Tube
- 1 - Inoculation loop (per plate)
- 150 mL - water
- 1 - pipette
- Petri Plates
- 1-sharpie

SAFETY WARNINGS

Be very careful when heating and handling LB Agar. Make sure the container lid is ajar when heating LB Agar mixture in the microwave. If the mixture begins to boil up, turn off the heat to let the bubbles settle. Use a heat resistant material to handle the hot flask. It is very hot and is dangerous to touch. Allow the agar to cool down from hot to a barely warm temperature before handling.

DISCLAIMER:

DISCLAIMER – FOR INFORMATIONAL PURPOSES ONLY; USE AT YOUR OWN RISK

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This protocol will assist you in making the agar plates for the CRISPR experiment. As you may have discovered in your prelab, the agar provides the bacteria with the nutrients needed to grow. Once your plates are made you may use an inoculation loop to streak them with bacteria. Over time your bacteria should grow and you will see little specs of colonies formed by your culturing. You will have to be very attentive to your plates for a successful experiment.

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Prep:15
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BEFORE STARTING

Additional protocols for clarity:

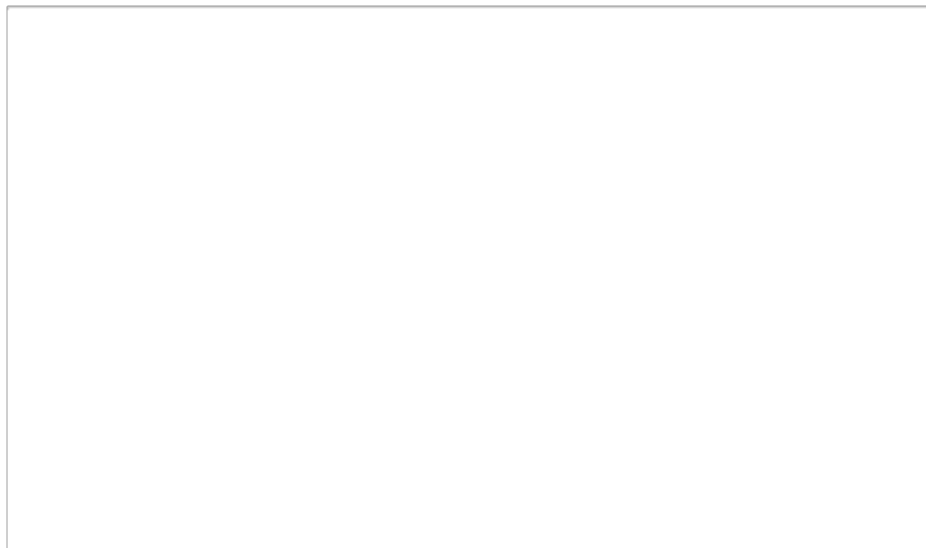
[Making Agar Plates](#)

[Pouring the Plates](#)

[Streaking the Plates](#)

1 To prepare for this lab, start by putting on your PPE gear, gathering your materials, and watching this video. ⌚00:15:00

Watch this video before boiling the agar.



2 Add agar and water to erlynmeyer flask. ⌚00:10:00

To calculate the amount of LB media use the following equations:

$$\frac{7g \text{ Agar}}{20 \text{ Agar plates}} = \frac{g \text{ of Agar needed}}{\text{Agar plates needed}}$$

$$\frac{(7 g \text{ Agar})(\text{Agar plates needed})}{20 \text{ Agar plates}} = g \text{ of Agar needed}$$

The above equation will allow you to have a great sense of the amount of Agar needed to prepare the plates. Weigh the correct amount of agar onto your scale. For the amount of water needed for plates, you want to have about 20 mL of water more than you need. This is because when you heat it up in the microwave, some of the liquid may escape. Each plate can take about 10 mL of agar . So if you are trying to make 5 LB plates, you'll want 1.75 g of agar and roughly 70 mL of water. The weigh paper to create a funnel allowing you to transfer the agar to the 250 mL flask. Place aluminum foil over the opening of the flask and swirl until agar seems well spread out.

3 Dissolve the solution. ⌚00:02:30

Place the flask in a boiling water bath for thirty second intervals. Use your choice of heat resistance material to handle the hot flask. Swirl it around and check for homogenization. Continue heating until desired consistency is obtained. Refer back to the video if necessary.

4 Plate agar. ⌚00:15:00

Have plates (aka:petri dishes) ready to pour. Do not open them before. Label plates to distinguish between the regular LB agar plates and the LB strep\kan\arabinose agar plates used for antibiotic resistance testing. Label the time time, date, and order to record growth in your protocols chart. Leave the flask on a heat resistance surface at room temperature for at least 10 minutes. You must make sure you pour the agar when it has cooled down but before the point of solidifying. Pour one plate at a time. You only need to pour the plates until the bottom is evenly covered.

5 Repeat. ⌚00:30:00

Wash the flask (use hot water if necessary) and repeat with LB Strep/Kan/Arab Agar.

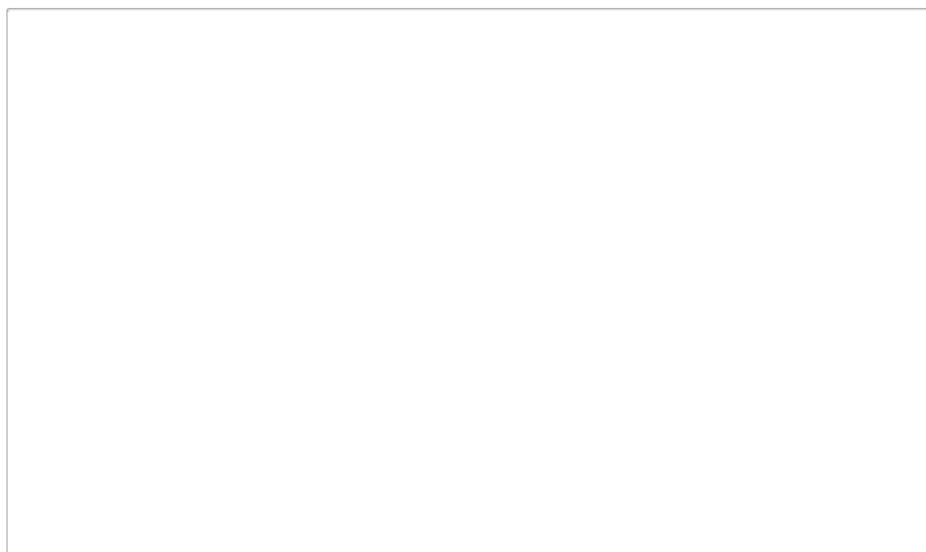
6 Allow for cooling. ⌚ 01:30:00

Allow plates to cool for 1.5 hours.

7 Store upside down.

This can be done in a safe room temperature atmosphere or the refrigerator for 4-24 hours.

8 Streak the LB agar plate with E coli. ⌚ 00:00:00



Add 100 uL of sterile water to Non-pathogenic E. coli bacteria Freeze Dried Tube (DH5α). Use inoculation loop to streak 2-3 LB-only plates. Store upside down for 12-48 hour in room temp (preferably a warm room to increase growth rate). Once you have noticed colonies growing in your petri dish you may move onto part 2. If you do not see growth within 3 days, repeat this step with the other unused LB-only plates.