LB Agar Plates

Caroline LaManna

Abstract

The following protocol is for making LB agar plates for the purpose of bacterial selection (500mL of LB agar makes about 25 LB agar plates). Please see the Addgene website for additional details.

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Protocol

Step 1.
Weigh out the following into a 1L Erlenmeyer flask: 5g NaCl

AMOUNT
5 g Additional info:

REAGENTS
Sodium chloride View by P212121

NOTES
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If your lab has pre-mixed LB agar powder, use the suggested amount instead of the other dry ingredients above.

Step 2.
5g Tryptone

AMOUNT
5 g Additional info:

Step 3.
2.5g Yeast Extract

AMOUNT
3 g Additional info:

Step 4.
7.5g Agar

AMOUNT
8 g Additional info:

Step 5.
add (dH2O) to 500mL

Step 6.
Swirl to mix

NOTES
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The contents do not have to be completely in solution, but any powder left on the sides of the flask will caramelize on the glass during autoclaving.

Step 7.
Cover the top of the flask with aluminum foil and label with autoclave tape.

Step 8.
Autoclave on the liquid setting for 20 minutes or according to your autoclave's specifications.

Step 9.
After removing the solution from the autoclave, allow the agar solution to cool to 55°C.

NOTES

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This can be done by placing the flask in a 55°C oven or water bath, as this will hold the temperature and it can be left unattended for some time.

Step 10.
Add the appropriate amount of desired antibiotic to the solution (500µL if you are using a 1,000x antibiotic stock) and swirl to mix.

NOTES

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If the solution is too warm when you add the antibiotic, it may degrade the antibiotic or damage the petri plates. If the solution is too cool, it may solidify before you have time to pour the plates.

Step 11.
Pour 20mL of LB agar per 10cm polystyrene Petri dish.

NOTES

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When pouring plates, keep your bench area sterile by working near a flame or bunsen burner.

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Pour slowly from the flask into the center of the petri dish. When the agar has spread to cover about 2/3 of the dish stop pouring and the agar should spread to cover the entire plate. You may need to tilt the plate slightly to get the agar to spread out completely. If you pour in too much, the plate will be fine, but it will reduce the number of plates you can make per batch.

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If bubbles are introduced during the pouring, these can be removed by quickly passing the flame of an inverted bunsen burner over the surface of the plate. Be careful, if you leave the flame too long it will melt the petri dish. Also be careful not to burn yourself.

Step 12.
Place the lids on the plates and allow them to cool for 30-60 minutes (until solidified)

DURATION
01:00:00

Step 13.
Invert the plates and let sit for several more hours or overnight.

DURATION
12:00:00

Step 14.
Label the bottom of plates with antibiotic and date and store in plastic bags or sealed with parafilm at 4°C.

NOTES

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Storage time will vary depending on antibiotic added, but plates are generally good for 1-2 months.