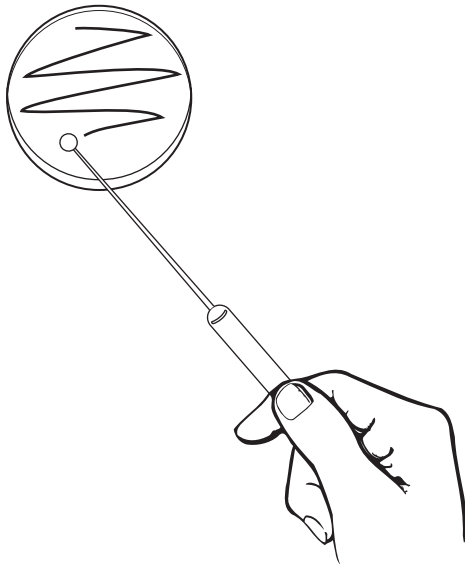


Instructions for Bottled Media



Caution:

Do not attempt to melt bottled media in the microwave. It is not possible to vent the bottle without contaminating the media. Heating the bottle in the microwave may cause pressure buildup, thereby creating an explosion hazard. For your safety, please follow the recommended procedures below.

Preparing the Medium

1. Fill a small pot halfway with water. (Or, use a large pot for several bottles.)
2. Place the bottle(s) of medium unopened inside the pot. Loosen the cap slightly. Adjust the water level to make sure it is even with the level of agar in the bottle(s).

Cautions: Removing the cap from the bottle before pouring the plate will contaminate the medium.

Do not place a cold bottle into boiling water! It may cause the glass bottle to shatter.

3. Heat the pot until the medium has melted completely. It may take up to 30 minutes to completely melt the medium.
4. Using an oven mitt or a pot holder, remove the bottle(s) from the water and set on a heat resistant surface.
Caution: In addition to being hot, the bottle(s) may be slippery. Please use extreme caution.
5. Allow the bottle(s) to cool to approximately 45° C before pouring plates. At that point, the medium will still be liquid but the bottle(s) will not be too hot to handle.

Note: Do not place the bottle(s) in an ice bath or refrigerator in an attempt to speed the cooling process.

Preparing the Work Area

1. Sanitize and disinfect the work area by cleaning with 70% alcohol or a bleach solution. Allow the area to air dry.
Note: A bleach solution may be prepared by adding 4 parts water to 1 part household bleach.
2. Wash and dry your hands thoroughly.

Pouring the Plates

1. Place the agar plates on the prepared work surface.
2. Remove the cap of the bottle you are going to use. Sterilize the mouth of the medium bottle by passing it through the flame of a bunsen burner or by wiping with an alcohol prep.
3. Lift the cover of a plate just enough to pour in the medium.
4. Pour a small amount of agar medium into the plate. Use just enough to cover the bottom of the dish. Each bottle will pour approximately five 100-mm plates.

Note: Pour only one plate at a time to minimize contamination. Hold the agar bottle tilted at close to a 45° angle until all the medium in the bottle has been used to pour plates. Never set the bottle down or hold it upright during the pouring process because this increases the potential for contamination.

5. Immediately close the plate cover. **Remember:** Do not set the bottle down or hold it upright.
6. Follow the same steps to pour the remaining plates.
7. Allow at least 30 minutes for the plates to set up.
8. Dispose of the empty bottles (See Disposal section.)

Inoculating the Streak Plates

Prior to inoculating the streak plates, it is important that the agar surface be smooth and moist; however, excessive moisture is undesirable since it can cause the bacterial colonies cultured on the plate to merge. Be sure the agar is allowed to set for at least 30 minutes before establishing cultures on the plates.

Note: For a standard inoculation, streak in the simple zigzag pattern shown in Figure 1, using either a sterile swab or wire loop. If you are trying to isolate types of bacteria on a plate, use an isolation streaking pattern, such as the one shown in Figure 2. Isolation streaking requires a wire loop, which must be flamed between each of the streaks on a plate. In isolating bacteria, each successive streak creates a more “diluted” population on its section of the plate.

1. Place the plates on the clean work surface.
2. Use either a fresh, sterile, cotton swab, just removed from its package,

or a thoroughly flamed wire loop.

Note: You must use a new, STERILE swab or a flame-sterilized loop for each plate.

3. Rub the swab or loop over or place it in the material to be cultured.
4. Lift the lid of an agar plate.
5. Streak the plate. Use either a simple zigzag streak (Figure 1) or an isolation streaking method (Figure 2). Quickly replace the lid after making each streak. **Remember:** For isolation streaking, a wire loop must be used.

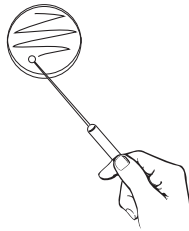


Figure 1. Simple streak (use sterile loop or swab)

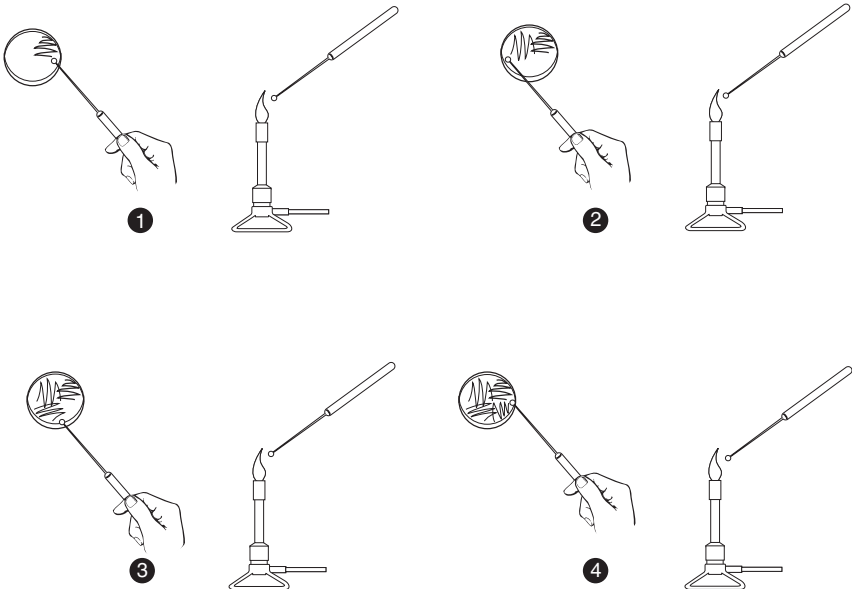


Figure 2. Isolation streaking (use metal loop only and flame it between streaks)

6. Incubate the plates.

Notes: For room-temperature incubation, place the plates agar-side up. If using an incubator, invert the plates and incubate them at 30–37° C. The specific time and temperature required for incubation depends on the medium selected.

7. Sterilize wire loops or dispose of used swabs. See Disposal section.

Storage

Unopened medium bottles may be stored under refrigeration for up to 1 year or at room temperature for up to 6 months.

Disposal

For used plates, medium, bottles, and swabs:

Method 1

1. Double bag all used or contaminated materials with autoclavable disposal bags.
Note: “Contaminated” refers to any package whose original seal was broken, even if the contents were not used.
2. Vent the disposal bag to allow steam to penetrate the inner contents.
3. Place in an autoclave at 121° C, 15 psi for 45 minutes.
Note: Time may vary from state to state. Always check with the public health service in your state to determine the requirements for decontamination.
4. Indicate on the bag with special, autoclave indicator tape that the enclosed sample has been autoclaved.
5. Dispose of in an appropriate waste receptacle.

Method 2

1. Soak the used or contaminated material in a bleach solution (1 part household bleach:3 parts water) for 24 hours.
2. Place the materials in the disposal bag and seal it.
3. Incinerate the bag.

For wire inoculating loops:

Flame the wire in a bunsen burner to sterilize it.

Ordering Information

For petri dishes we recommend:

74-1350 Sterile, disposable, polystyrene petri dish, 100 × 15 mm

For sterile swabs we recommend:

70-3032 Cotton-tipped sterile applicators (10 packages of 2 applicators each)

70-3033 Cotton-tipped sterile applicators (1 box of 100 packages)

For wire loops we recommend:

70-3060 Nichrome wire inoculating loop

Tips for Successful Cultivation

1. Avoid contaminants. Do not use nonsterile plates, swabs, or media.
2. Properly sanitize and disinfect the work area according to instructions.
3. Label and date each individual petri dish. This will make recordkeeping much easier.
4. Use the proper inoculation techniques for specific culture media in order to obtain the correct biochemical growth patterns or reactions.
5. Use the correct medium for cultivation.
6. Incubate under appropriate conditions.
7. Follow preparation and disposal procedures.

To order call:

1-800-334-5551 (US and Canada)

336-584-0381 (International)

For technical help call:

1-800-227-1150

www.carolina.com

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