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LB lite Growth Media Protocol

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We use this protocol and it's working

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Disclaimer

This protocol has been tested in a standard microbiology laboratory setting. Users should ensure aseptic techniques and appropriate safety measures when preparing and handling bacterial culture media.

Abstract

This protocol describes the preparation of LB lite broth, a relatively less nutrient-rich medium used for bacterial growth. The protocol includes the preparation of the solution using yeast extract and sodium chloride, mixing procedures, and autoclaving for sterilization. The expected result is a sterile, ready-to-use LB lite broth and solid media suitable for microbial culture.

Guidelines

- Ensure all glassware and equipment are sterile before starting.
- Use a weigh boat to prevent contamination when measuring dry ingredients.
- When adding distilled water, use a graduated cylinder for precise measurement.
- Always use a magnetic stirrer to dissolve components thoroughly before sterilization.
- Label the bottle properly with medium type, preparation date, and initials.
- Store the prepared LB lite broth and solid media (with agar) at 4°C if not used immediately.

Materials

Chemicals & Reagents

- Yeast Extract (5 g per liter) – Nutrient source
- Sodium Chloride (NaCl) (10 g per liter) – Osmotic balance
- Agar (for solid media) (15 g per liter) – For solidifying LB agar

Glassware & Lab Equipment

- Weigh Boat – For measuring dry ingredients
- Analytical Balance – For accurate weighing
- 1L Autoclavable Bottles – For media preparation and sterilization
- 1L Graduated Cylinder – For measuring distilled water
- Magnetic Stir Bar & Magnetic Stirrer – For thorough mixing
- Sterile Magnetic Rod – For removing stir bar
- Autoclave – For sterilization
- Pipettes and Tips – For accurate liquid handling (if necessary)

For LB Agar Plate Preparation

- Petri Dishes – For pouring LB agar plates
- Bunsen Burner or Biosafety Cabinet – For sterile work
- Sterile Flask or Media Bottle – For storing molten agar before pouring

Sterility & Safety Supplies

- Autoclave Tape – To monitor sterilization
- Gloves & Lab Coat – For safe handling of media and bacterial cultures
- Sterile Workbench or Biosafety Cabinet – To prevent contamination

Safety warnings

- Autoclave Warning: Never tightly close the bottle lid before autoclaving to prevent explosion due to pressure buildup.
- Sterility Warning: After autoclaving, perform all further steps inside a sterile biosafety cabinet to maintain sterility.
- Handling Hot Bottles: Allow the bottle to cool to ~50°C before handling or pouring to avoid burns.
- Disposal: Dispose of used media and contaminated materials following biosafety waste disposal regulations.

Ethics statement

This protocol involves standard microbiology laboratory practices and does not require ethical approval.

Before start

Prepare the autoclave by checking water levels and settings.
Ensure that all reagents (sodium chloride, yeast extract) are within expiration dates.
Wear appropriate personal protective equipment (PPE), including gloves and a lab coat.
Work in a clean environment to avoid contamination.
Pre-check the autoclave temperature and pressure settings before sterilization.

Procedures

1 **Gather Materials**
- Collect all necessary materials, including weigh boat, sodium chloride, yeast extract, 1L bottles and Beaker, stir bar, graduated cylinder, and a magnetic rod.

2 **Weigh Sodium Chloride**
- Place a sterile weigh boat on a tared scale.
- Weigh 10 g of sodium chloride and transfer it to a 1L Beaker.

3 **Weigh Yeast Extract**
- Using the same weigh boat, weigh 5 g of yeast extract and transfer it to the 1L Beaker containing sodium chloride.

4 **Measure and Add Distilled Water**
- Using a graduated cylinder, measure 1 liter of distilled water and pour it into the 1L bottle.

5 **Mix the Solution**
- Place a sterile stir bar into the Beaker and mix the contents thoroughly using a magnetic stirrer.
- Once mixed, remove the stir bar using a sterile magnetic rod and tranfer the solution into 1L autoclave bottle.

Note: Use ~400 mL of DI water in a 1L beaker to dissolve the base media components thoroughly. After transferring the solution to the 1L media bottle, rinse the beaker with ~100 mL of DI water to capture any remaining constituents. Repeat this rinsing process multiple times until all media components have been fully transferred, ensuring complete dissolution and accurate media composition.

6
- **Prepare for Autoclaving**
- Secure the lid loosely and seal the bottle with autoclave tape to prevent pressure buildup.

7 **Sterilization**
- Autoclave the bottle at 121°C for 15 minutes at 15 psi to ensure sterility.

8 **For LB Agar Preparation**
- If preparing LB agar, add 15 g/L of agar before autoclaving.
- Keep the stir bar in the bottle to aid mixing when pouring agar plates.

Protocol references

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