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Glycerol Stock Solution Preparation for Long-Term Bacterial Storage

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We use this protocol and it's working

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Disclaimer

This protocol has been validated under controlled laboratory conditions. Users must follow biosafety guidelines when handling bacterial cultures. Proper aseptic techniques and PPE should be used to minimize contamination. The authors are not responsible for improper execution or handling errors.

Abstract

This protocol describes the preparation of glycerol stock solutions for long-term bacterial preservation at -70°C or -80°C . Using a cryoprotectant (glycerol) prevents cell damage due to ice crystal formation. The process includes bacterial culture growth, centrifugation, mixing with glycerol, and freezing for long-term viability.

Guidelines

- Always work under a BSC to maintain sterility.
- Glycerol concentration should remain at 40% final volume to protect cells during freezing.
- Avoid repeated freeze-thaw cycles to prevent cell damage and maintain viability.
- Screw-cap tubes are preferred for freezing at -80°C , as flip-cap tubes may pop open.
- Ensure labels are protected with clear tape to prevent damage during long-term storage.

Materials

Reagents & Solutions

- LB Broth – For bacterial culture growth
- 80% Glycerol Solution – Cryoprotectant for cell preservation

Consumables & Equipment

- Bacterial Culture in a 250 mL Erlenmeyer Flask – Overnight-grown bacterial suspension
- 100-1000 µL Pipette – For precise liquid handling
- Autoclaved 1250 µL Pipette Tips – Sterile handling of bacterial suspension
- Autoclaved 2 mL Flip-Cap Tubes – For temporary storage (not preferred for freezing)
- Autoclaved 2 mL Screw Cap Tubes – Cryogenic vials for long-term storage
- Biological Safety Cabinet (BSC) – Ensuring sterile working conditions
- Gloves – For aseptic handling
- Scissors – For cutting tape for labeling
- Ethanol Lamp & Lighter – Maintaining sterility in BSC
- Ethanol Spray Bottle – For sterilization
- KimWipes® – For wiping surfaces
- -70°C or -80°C Freezer – For bacterial preservation

Safety warnings

- ! ▪ Glycerol is viscous and should be pipetted carefully to avoid contamination.
- Use PPE when handling bacterial cultures to minimize the risk of contamination.
- Ensure ethanol lamps are safely handled and turned off after use.
- Store glycerol stocks immediately after preparation to prevent degradation.

Ethics statement

This protocol involves the handling and long-term preservation of bacterial cultures under biosafety guidelines. It does not involve animal or human subjects. However, researchers must adhere to institutional biosafety regulations (BSL-1 or BSL-2, depending on the bacterial strain used). Proper aseptic techniques, personal protective equipment (PPE), and sterile handling practices should be followed to prevent contamination and ensure laboratory safety.

Researchers must dispose of biological waste, including bacterial cultures and contaminated materials, according to institutional and regulatory guidelines. If handling pathogenic bacteria, approval from the institution's biosafety committee may be required.

Before start

- Prepare all materials in advance and autoclave necessary consumables.
- Ensure the ethanol lamp is properly filled and functioning before starting work.
- Label all cryogenic tubes with bacterial strain, date, and relevant details before transferring cultures.

Procedures

1 Labeling and Workspace Sterilization

1. Label all flip-cap and screw-cap tubes with:
 - Genus and species of bacteria
 - Tube number
 - Date and researcher name
 - Indicator for opened/unopened tubes (helps track sterility)
2. Wrap labels with clear scotch tape to prevent damage.
3. Transfer all materials to the BSC and sterilize the working surface using ethanol spray and KimWipes®.
4. Light the ethanol lamp inside the BSC for additional sterility.

2 Bacterial Culture Growth

1. Using a sterile cell spreader, pick a single colony from an isolated bacterial plate.
2. Inoculate a 250 mL Erlenmeyer flask containing 50 mL of LB broth with the selected colony.
3. Incubate at 28°C with shaking at 200 rpm for 24 hours.
4. Visually inspect the culture for turbidity, confirming bacterial growth.

3 Glycerol and Bacterial cells Preparation

1. Pipette 800 µL of sterile 80% glycerol solution into a 2 mL cryogenic screw-cap vial.
2. Transfer 1.5 mL of the bacterial culture into a sterile 2 mL Eppendorf tube.
3. Centrifuge at maximum rpm (12,000–14,000 x g) for 1–3 minutes to pellet bacterial cells.
4. Discard the supernatant carefully without disturbing the pellet.
5. Add another 1.5 mL of LB broth containing bacterial cells, centrifuge again, and discard the supernatant.
6. Repeat the centrifugation step until a sufficient bacterial pellet is obtained.

4 Mixing Cells with Glycerol

1. Resuspend the bacterial pellet in 800 µL of sterile 80% glycerol (prepared in Step 9).
2. Ensure a 1:1 ratio of glycerol and bacterial suspension to achieve a final glycerol concentration of 40%.
3. Gently vortex the tube to ensure thorough mixing without excessive agitation, which may damage bacterial cells.
4. Incubate the mixture at room temperature for 30 minutes to allow the glycerol to penetrate the cells and protect them from freeze stress.

Note 1: The 30-minute incubation step ensures proper cryoprotection, reducing the risk of cell damage during freezing.

Note 2: It is not recommended to prepare multiple bacterial strains at the same time to prevent cross-contamination and maintain the integrity of each glycerol stock. If multiple



strains need to be prepared, work with one strain at a time, thoroughly sterilizing the workspace between strains.

5 Freezing and Storage

1. After incubation, proceed to freezing the glycerol stock at -70°C or -80°C.
2. Ensure the stocks remain frozen at all times.
3. To retrieve bacteria, scrape frozen stock with a sterile loop or spreader instead of thawing the tube.

6 Expected Results

- Well-preserved bacterial cells in frozen storage, maintaining viability for long-term use.
- Cells remain viable for years when stored properly without repeated freeze-thaw cycles.
- Successful bacterial recovery when streaked from frozen stock onto fresh LB agar plates.

Protocol references

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