

Rev. 8/06/26

General Guidelines for Freezing and Long-Term Storage of Microbial Cultures with CLEARStable

CLEARStable is an efficient system designed for the long-term freezing and storage of microbial cultures, ensuring stability while avoiding the need for freeze-drying or frequent sub-culturing.
The system includes a cryobox containing 80 sterile cryotubes, each containing 20±1 porous beads immersed in 1±0.1 ml of cryoprotectant solution with glycerol. This format is ideal for preserving a various range of microorganisms, including bacteria, spore-forming bacteria, fungi, molda and yeasts.

A dry version (without cryoprotectant solution) is also available.
CLEARStable enables safe freezing and storage at -20°C, -80°C, or in liquid nitrogen vapor, maintaining both viability and genetic stability according to specific storage requirements.

Usage Procedure

For dry version only:

Before starting the preservation procedure, add a suitable cryoprotectant (selected by the operator) according to the specific microorganism or strain to be preserved.
Ensure that the porous beads are completely immersed in the cryoprotectant solution before proceeding.
Once the cryoprotectant has been added, continue with the standard instructions for use described below.

1. Preservation of the Microbial Strain:

1.1. Preparation of the microorganisms

- For greater efficiency, ensure that the culture (either liquid suspension or plate) is prepared the day before use, allowing it to reach the logarithmic growth phase (typically 20-24 hours, depending on the microorganism).

1.2. Preparation of the Cryotube

- Remove a sterile cryotube from its packaging, ensuring sterility is maintained.
- Label the cryotube with the microbial strain name, date, and any other relevant information.

1.3. Inoculation

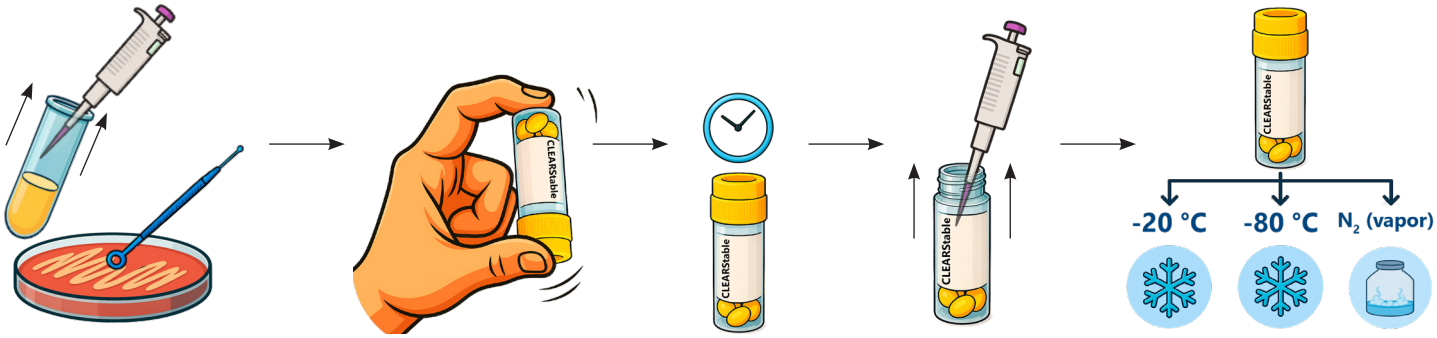
- From liquid suspension: using a sterile inoculation loop or pipette, transfer approximately 100-200 µl of actively growing culture into the cryotube.
- From plate Culture: Take a sterile bead from the cryotube, gently streak it across the inoculated plate containing microorganisms. Ensure proper adherence of the microorganisms to the bead before transferring it back into the cryotube. Alternatively, you can also pick colonies directly from the plate and transfer them into the cryotube.

1.4. Mixing and Distribution

- Cap the cryotube and gently invert it several times to ensure the beads are fully immersed in the cryoprotective solution and that the microorganisms are evenly distributed. **Do NOT vortex**.
- Let the vial stand for 30-60 seconds.
- Using a sterile pipette, remove the excess cryoprotective solution, leaving the beads as free of liquid as possible.
- Close the vial finger tight.

1.5. Freezing the Cryotube

- Immediately store the inoculated cryotube in a ***-20°C freezer*** for short-term storage or a ***-80°C freezer*** for long-term storage, or in the ***vapor phase of nitrogen*** for optimal preservation, ensuring the freezer is well-maintained and monitored to maintain a constant temperature.



2. Retrieval of the Microbial Strain:

2.1. Removal of the cryotube from the nitrogen vapor phase or the freezer

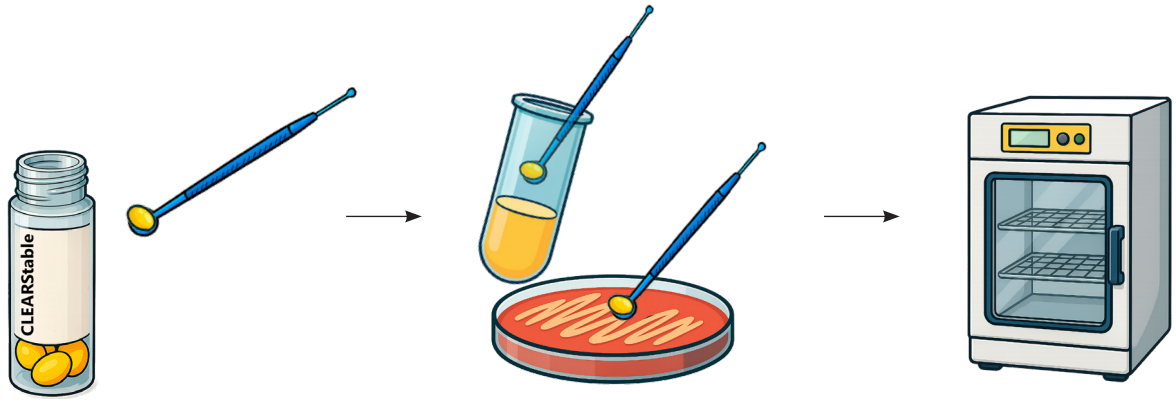
- When necessary, promptly remove the cryotube from storage.

2.2. Removal of a Bead

- Open the cryotube and, using sterile tweezers or a similar tool, (e.g., Microloops ref BSV120 from Biosigma), remove a frozen bead.
- Immediately close the cryotube and return it to the freezer to minimize temperature fluctuations.

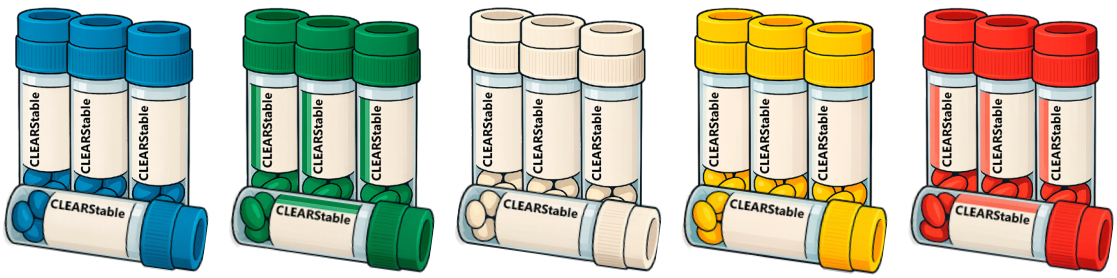
2.3. Thawing and Inoculation

- Transfer the frozen bead into a fresh, appropriate culture medium (e.g., nutrient broth or agar plate). If using a liquid medium, immerse the bead into the broth; if using a solid medium, gently streak the bead across the surface.
- Incubate at the optimal temperature for the recovery and growth of the microorganisms.
- Safely discard the bead after use, according to the established guidelines for contaminated material disposal.



Precautions and Warnings:

- **Sterility**: Always work under sterile conditions to prevent contamination of microbial cultures.
- **Personal Protective Equipment (PPE)**: Wear appropriate PPE throughout all handling procedures.
- **Temperature Control**: Minimize temperature fluctuations, as they can compromise microorganism viability. When retrieving a bead, avoid thawing the remaining beads. A cryoblock can be utilized to maintain a stable temperature. Keep the cryoblock frozen and place the vial inside it when outside the freezer to preserve organism viability.
- **Vial Integrity**: Do not use CLEARStable if the vial appears turbid, indicating possible contamination, or if there are signs of leakage.
- **Storage Limitations**: Store organisms at the appropriate temperature specific to each strain, following the recommended guidelines for their type.
- **Certificate of Analysis**: For each production batch, a certificate of analysis is provided. A sterility test and performance test are conducted to ensure product quality and effectiveness.
- **Product Suitability**: While CLEARStable has been validated with standard collection strains, users should evaluate its effectiveness for their specific strains.
- **Color Variants**: CLEARStable provides a variety of cryovials featuring caps that coordinate with the color of the beads. Choose from an array of colors, including white, red, blue, yellow, green, or a blend of these options. Each vial is filled with the same effective cryoprotectant, guaranteeing consistent performance regardless of the color.
- **Flexible options**: Available with or without cryoprotectant, and with 1D or 2D barcoding.
- **Storage**: Before use, unused CLEARStable may be stored between 4°C and 30°C.



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