OPS DIAGNOSTICS
LABORATORY PRODUCTS FOR SAMPLE PREPARATION

Bacterial Freezing Kit

Product No. CRY 162-03
Store at Room Temperature
STERILE – HANDLE ASEPTICALLY

Bacterial Freezing Kits are a simple and convenient tool for freezing bacterial cultures. Each tube contains sterile glycerol and when mixed with bacterial culture broth, creates a solution that helps to protect bacterial cells during freezing. The use of 15% glycerol has been documented as a key ingredient in preserving bacteria for over half a century. Though not all bacteria require a cryoprotective agent, most have better survival rates with glycerol.

As bacterial cultures typically have high densities of cells (e.g., 1 x 10^9/ml), the process for cryopreserving cells can be simple, though more complex methods can be followed. A basic procedure simply involves mixing culture broth with the glycerol solution in the tube prior to freezing. Alternatively, cells which are frozen at a controlled rate, of usually 1˚C/min, have been shown to have higher survival rates. Controlled rate freezers exist for this type of processing, but they are expensive and usually not necessary for bacterial cultures.

Before freezing any precious culture with this or any other cryopreservative, test a small portion of the culture first to ensure that this glycerol solution will adequately protect the bacteria from deleterious effects of freezing. Not all bacteria behave the same way to freezing methodologies.

Each package of Bacterial Freezing Kit contains:

2 Cryogenic Storage Boxes
162 Cryogenic Tubes with Glass Beads and 225 µl Sterile 30% Glycerol
2 Tube Extractors (for easily pulling tubes from the storage boxes)
2 Sheets of Inkjet Labels
1 Set of Instructions

Freezing Procedure

1. Under sterile conditions, remove the cap from a Bacterial Freezing Tube. Add 225 µl of fresh culture broth, recap and shake or briefly vortex to mix.
2. Repeat for remaining tubes.
3. Label the tubes with organism, name, date, etc. You may write directly onto the tubes with a permanent marker, or use the small inkjet printer labels. These labels will stick on the tubes at cold temperatures and are resistant to alcohol when printed with an inkjet printer. If you would like to handwrite on the labels, a permanent marker works best. A template for the inkjet labels can be found at:

   www.opsdiagnostics.com/bacterial_freezing_kit_p538.html

4. Place the tubes in the freezer. Generally a colder freezer will prolong the life of the stored cells.

Reactivating Cells

1. To reactivate cells, remove the tubes from the freezer but **DO NOT THAW**. Tubes can be placed in ice or in a cryogenic carrier to keep frozen. **USE ASEPTIC TECHNIQUE** when handling the tubes. Remove the cap and loosen a bead using a sterile micropipette tip. Pour the bead onto the surface of a suitable and sterile agar plate. Roll the bead around to spread bacteria over the plate. Place the Bacterial Freezing Tube back into the freezer before it thaws. Repeated freezing and thawing of cells, and many other biomolecules, can lead to complete loss of viability.
2. Incubate the plate at a suitable temperature.
3. For older cultures that have had significant die off, thaw the tube and draw off the glycerol and transfer to the surface of an agar plate. Incubate at an appropriate temperature.

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