

## Bacterial Freezing Medium

Product No. CRY 125-04

Store at Room Temperature

**STERILE – HANDLE ASEPTICALLY**

Bacterial Freezing Medium is a simple and convenient way to freeze bacterial cultures. Each bottle contains a sterile glycerol based medium that helps to protect bacterial cells during freezing. The use of glycerol is well documented as a key ingredient in preserving bacteria during freezing 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 cell densities (e.g.,  $1 \times 10^9$  cells/ml), the process for cryopreserving cells is simple. A basic procedure involves pelleting cells and resuspending in an equal volume of Bacterial Freezing Medium prior to freezing. Alternatively, cells which are frozen at a controlled rate, of usually  $1^\circ\text{C}/\text{min}$ , have been shown to have higher survival rates. Controlled rate freezers for this type of processing are expensive and usually not necessary for most 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 medium will adequately protect the bacteria from deleterious effects associated with freezing. Not all bacteria behave the same way to freezing methodologies.

### Freezing Procedure

1. Culture cells to mid/late log phase in a suitable medium.
2. Spin down cells and decant the supernatant. Under sterile conditions, remove the cap from a Bacterial Freezing Medium.
3. Add on equal volume of Bacterial Freezing Medium to the tube.
4. Vortex to mix and resuspend the cells.
5. Label the tubes with organism, name, date, etc.
6. 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 using a sterile micropipette tip, scratch off medium from the surface and transfer to an agar plate.
2. 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/activity.
3. Incubate the plate at a suitable temperature.
4. For older cultures that have had significant die off, thaw the tube and draw off the medium and transfer to the surface of an agar plate. Incubate at an appropriate temperature.

### Additional Suggestions

An additional step to help simplify the reactivation process would be to add 3 mm or 4 mm sterile glass beads to the medium prior to freezing. When taking samples from the tube, a bead can be chipped off with a sterile pipette tip and rolled onto an agar plate. This will protect the frozen culture from the thawing.

BAWG 4000-200-18

4 mm Silica Beads - Acid Washed