

PREPARATION OF FROZEN STOCK CULTURES

Bacteria can be stored for many years in media containing 15% glycerol at low temperature without significant loss of viability.

1. Inoculate a culture flask containing 5-10 ml of liquid medium with a single bacterial colony and grow the culture overnight.
2. Transfer 3.4 ml of the overnight culture to a sterile vial containing 0.6 ml of sterile glycerol. Place the cap on the vial and mix the contents thoroughly by vortexing.
 - 2a. For using microfuge tubes, use:
0.15 ml glycerol + 0.85 ml culture
or
0.3 ml 50% glycerol + 0.7 ml culture
3. The glycerinated cultures can then be stored at -20°C for a few years without loss of viability. Alternatively, the glycerinated suspension can be stored at -70°C . In this case, viable bacteria can be recovered many years after the initial freezing. Viable bacteria can be recovered by simply scratching the surface of the frozen stock with a sterile platinum loop or wire. The frozen suspension can then be returned to the freezer. Several vials of each strain should be frozen.