

# SOP M016

## Freezing Protocol in Glycerin for Beads

Bacteria can be frozen using a solution of 15% glycerol. The process is simple and requires screw cap microfuge tubes and sterile glycerol. The glycerol is diluted to 30% so that it is easy to pipette. Equal amounts of 30% glycerol and culture broth are mixed, dispensed into tubes and then frozen. A kit is available ([Bacterial Freezing Kit](#)) through OPS Diagnostics that simplifies the preparation of sterile tubes for this process.

### Protocol

- Prepare a solution of 30% glycerol (v/v) by mixing 30 ml of glycerol with 70 ml of water. Transfer the solution to a screw cap glass bottle and sterilize by autoclaving at 121°C for 15 min. Loosen the cap during autoclaving.
- Aliquot 500 µl of sterile 30% glycerol into sterile 2 ml sterile microfuge tubes containing [4 mm glass beads](#)\*.
- Add 500 µl of bacterial culture to the tube and mix with the glycerol using a vortex mixer.
- Label the tube with the organism name, strain, date, etc.
- Place the tube in the freezer and record its location.
- To activate bacteria, only a couple of beads need to be removed from the tube. The tube doesn't need to be thawed. Open the tube and loosen a couple of beads using a sterile pipette tip. Pour the loose beads onto an agar plate and roll them around. Colonies that develop should be re-streaked. Replace the tube into the freezer immediately. If the culture thaws, do not re-freeze it as cells are typically very sensitive to freezing and thawing. Discard the thawed culture appropriately.

\* We prefer tubes that are screw cap and have caps with O-rings. One option is to fill the tube 1/3 full with 4 mm glass beads ([product number BAWG 4000-200-18](#)) before sterilizing. When retrieving bacteria, remove or chip out one bead onto an agar plate and roll around to disperse the bacteria. This avoids thawing the entire stock.

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Prepared By

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