

## Preserving yeasts at -80 °C or at -150 °C

### Materials

1. Substrates: YPD (Yeast extract peptone dextrose broth); PDB (Potato Dextrose broth); SDB (Sabouraud Dextrose broth).
2. Glycerol 100%
3. Sterile CryoTube Vials 2 mL screw-capped (-80 °C)
4. Sterile CryoTube Vials 1 mL screw-capped with barcode (-150 °C)
5. Cryo 1°C Freezing Container “Mr. Frosty” (Nalgene Labware) cooling rate 1 °C/min
6. Safety equipment: should include cryogloves (cold resistant), face shield, forceps, and oxygen monitor.

### Procedure

1. Inoculate a loop of biomass grown on the surface of a Petri dishes containing a suitable medium (e.g. YPD agar) into a test tube containing 3 ml of corresponding liquid medium.
2. Prepare a sterile stock solution 100% glycerol (autoclave for 20 minutes).
3. Grow a culture of the strain to be preserved on a specific substrate depending on the genus/species for 1-3 days (or however long it takes to obtain at least 0.8-1 OD<sub>600</sub> per ml).
  - 1) Collect actively grown cells from the fresh culture with a pipette.
  - 2) Prepare cryo tubes by adding glycerol 100% to obtain a concentration of 15% final volume.
  - 3) Transfer the suspension into the cryo tube containing the glycerol solution and mix thoroughly.
  - 4) Store in cryobox at -80°C freezer

5) Freeze in -150 °C freezer or over liquid N<sub>2</sub> (do not submerge in liquid N<sub>2</sub>).

We at ITEM use some special racks for freezing: Cryo 1°C Freezing Container “Mr. Frosty”

- 1) Allow at least 1 h for the cells to equilibrate in the glycerol
- 2) Pre-cool the tube at 4 °C for about 10 min
- 3) Put in the -80 °C freezer special racks able to freeze at a rate of 1 °C/minute after 3 hours transfer the cryovials into storage racks at (-150 °C) in the ultrafreezer or in vapour of liquid nitrogen.

### Revitalization of frozen yeast culture

Warm the cryovial rapidly by immersion in a water bath at 37 °C and leave them there until the ice is completely thawed (thawing—reactivation of cultures). Remove immediately on completion of thawing and do not allow it to warm up to the temperature of the bath.

1. Open aseptically the cryo-vial by avoiding the complete melting of the sample and collect 10-50 microlites by using a sterile pipet tip.
2. Inoculate the sample into a fresh Petri plate or into a tube containing 5 ml of a medium (SDA, YPD broth) and incubate the culture at 25-30°C under agitation for 1-4 days.
3. Check the yeast viability, the morphology and the purity of the colonies onto appropriate agar medium.