

Protocol for Cryopreservation of different bacterial strains.

Protocole code: Cry

Materials:

- Culture media (Broth).
- Sterile glycerol.
- Sterile 1.5 mL microcentrifuge tubes.
- -80°C freezer./-20°C freezer.
- Bacterial strain.
- Incubator with and without shaking.
- Micropipettes.
- Micropipette tips.

Procedure:

Day 1:

1. Inoculate the culture media with the bacterial strain you need to cryopreserve.
2. Incubate in shaking at the optimal temperature and oxygen availability for the time the strain need to reach a late exponential phase:
 - i. For *Escherichia coli*: 37°C, 24 h at aerobic conditions.
 - ii. For *Lactobacillus casei* ATCC 334TM, 37°C in microaerophilic conditions.
 - iii. For *Clostridium difficile*, 37°C at anaerobic conditions.

Day 2:

1. Take 800µL of the strain culture and add it to a sterile microcentrifuge tube.
2. Add 200µL of sterile glycerol to the previous tube.
3. Store at -80°C for at least one night or up to two years or store at -20°C for at least one night or up to three months.