Freezing Cells and Thawing Frozen Cells

Lab 10

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Cryopreservation

- Cryopreservation is a method of preserving biological tissue or cells at ultra low temperatures (-196°C).

- At such temperatures metabolism gets virtually halted.

- Storing cultured cells in liquid nitrogen with complete medium in the presence of a cryoprotective agent such as dimethylsulfoxide (DMSO) or Glycerol is the best method for cryopreserving cultured cells .

Cryoprotective agents

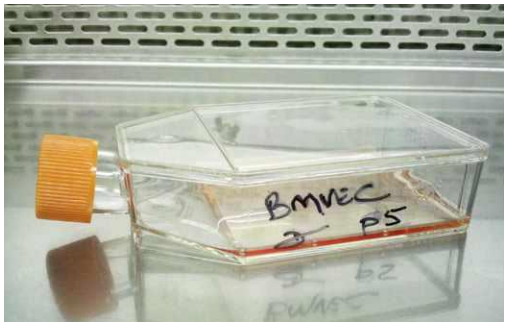
- reduce the freezing point of the medium and allow a slower cooling rate.
- greatly reducing the risk of ice crystal formation, which can damage cells and cause cell death.

Guidelines for Cryopreservation

- Freeze your cultured cells at a high concentration and at as low a passage number as possible. Make sure that the cells are at least 90% viable before freezing.
- Freeze the cells slowly by reducing the temperature at approximately 1°C per minute using a controlled rate cryo-freezer or a cryo-freezing container such as "Mr. Frosty,"
- Always use the recommended freezing medium. The freezing medium should contain a cryoprotective agent such as DMSO or glycerol.
- Store the frozen cells below –70°C.

- Always use sterile cryovials for storing frozen cells.
- Always wear personal protective equipment.
- All solutions and equipment that come in contact with the cells must be sterile.
- Use proper sterile technique and work in a laminar flow hood.

Materials

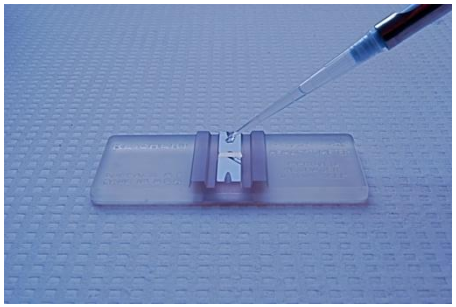


cultured cells

Complete growth medium

Cryoprotective agent (DMSO)

Sterile 15-mL or 50-mL conical tubes



hemacytometer

Sterile Cryovials

Controlled rate freezing apparatus

Or

Mr. Frosty Freezing Container

Liquid nitrogen storage container

For freezing adherent cells, in addition to the materials, you need:

- Balanced salt solution such as Dulbecco's Phosphate Buffered Saline (D-PBS).

- Dissociation reagent such as trypsin.

Protocol for Cryopreserving Cultured Cells

1. Prepare freezing medium and store at 2° to 8°C until use.

2. For adherent cells, gently detach cells from the tissue culture vessel following the procedure used during the subculture. Resuspend the cells in complete medium required for that cell type.

3. Determine the total number of cells and percent viability using a hemacytometer. According to the desired viable cell density, calculate the required volume of freezing medium.

4. Centrifuge the cell suspension at approximately 100–200 × *g for 5 to 10 minutes* Aseptically decant supernatant without disturbing the cell pellet.
Note: Centrifugation speed and duration varies depending on the cell type.

5. Resuspend the cell pellet in cold freezing medium at the recommended viable cell density for the specific cell type.

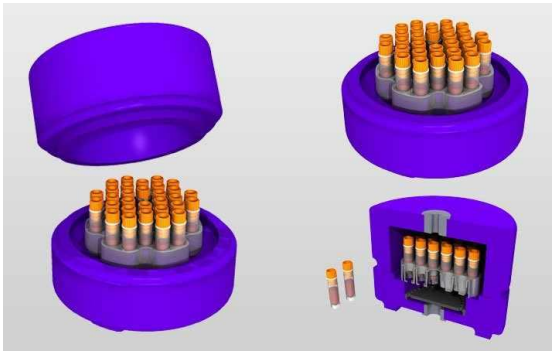
6. Dispense aliquots of the cell suspension into cryogenic storage vials. As you aliquot them, frequently and gently mix the cells to maintain a homogeneous cell suspension.

7. Freeze the cells in a controlled rate freezing apparatus, decreasing the temperature approximately 1°C per minute. Alternatively, place the cryovials containing the cells in an isopropanol chamber and store them at –80°C overnight.

8. Transfer frozen cells to liquid nitrogen, and store them in the gas phase above the liquid nitrogen.

Thawing Frozen Cells

Materials



Cryovial containing frozen cells

Complete growth medium,
pre-warmed to 37°C

sterile centrifuge tubes

Water bath at 37°C

70% ethanol

Tissue-culture treated flasks, plates, or dishes

Protocol for Thawing Frozen Cells

1. Remove the cryovial containing the frozen cells from liquid nitrogen storage and **immediately place it into a 37°C water bath.**

2. Quickly thaw the cells (< 1 minute) by gently swirling the vial in the 37°C water bath until there is just a small bit of ice left in the vial.

3. Transfer the vial it into a laminar flow hood. Before opening, wipe the outside of the vial with 70% ethanol.

4. Transfer the thawed cells **dropwise into the centrifuge tube containing the desired** amount of pre-warmed complete growth medium appropriate for your cell line.

5. Centrifuge the cell suspension at approximately 200 × *g for 5–10 minutes.*

6. After the centrifugation, check the clarity of supernatant and visibility of a complete pellet. Aseptically decant the supernatant without disturbing the cell pellet.

7. Gently resuspend the cells in complete growth medium, and transfer them into the appropriate culture vessel.

8. Label the flask, initial, date, and Incubate @ 5% CO2 incubator & 37°.

References

http://www.jove.com/video/2206/cryostor-cryopreservation-protocol-advertisement

http://www.youtube.com/watch?v=CCWrLUA6Qbg

http://www.invitrogen.com/site/us/en/home/References/gibco-cell-culture-basics/cell-culture-protocols/freezing-cells.html

http://www.invitrogen.com/site/us/en/home/References/gibco-cell-culture-basics/cell-culture-protocols/thawing-cells.html