Lonza

Tips for Successful Cryopreservation

Technical Reference Guide

The practice of keeping a stock of frozen cultures should be a part of any cell culture laboratory. Cell lines or clones should be stored frozen to protect against genetic changes or loss of cultures due to contamination or incubator failure. Storage of cells in liquid nitrogen allows for prolonged storage, of up to 10 – 12 years. Cells can be stored in either the liquid nitrogen phase (-180 °C to -195 °C) or in the gas phase (-140 °C to 145 °C). The gas phase may be the better choice as the liquid can seep into the cryovial and cause the vial to "explode" upon thawing.

Cells to be frozen should be free of contamination and in the log phase of growth. The freezing medium should contain 5 – 10% cryopreservative and 5 – 20% serum. Commonly used cryopreservatives are glycerol or dimethyl sulfoxide (DMSO). Glycerol and DMSO lowers the freezing point. A slow cooling rate (1 – 2 degrees/minute) allows water to move out of the cell before it freezes. This protects against mechanical injury from ice crystal formation.

General Procedure

Adherent Cells

1. Remove cell culture medium.
2. Wash monolayer with calcium or magnesium-free PBS.
3. Remove PBS.
4. Add trypsin solution (just enough to cover monolayer).
5. Monitor the detachment process carefully to avoid cell damage.
6. When cells begin to round up, add complete medium containing serum or a trypsin-inhibitor solution.
7. Perform a cell count.
8. Cells will be frozen at 5 x 10^6 to 1 x 10^8 cells/mL (cryovials generally are 1 – 2 mL in size).
9. Based upon the number of vials to be prepared and cell number needed, centrifuge the appropriate volume of the cell suspension.
10. Aspirate off the growth medium.
11. Resuspend in freezing medium at 5 x 10^6 to 1 x 10^8 cells/mL.
12. Place vials in automatic freezing unit. If no such device is available, place vials on ice for about 1 – 2 hours, move to -20 °C freezer for several hours, next move -70 °C freezer for several hours, and finally into liquid nitrogen storage.

Suspension Cells

1. Perform a cell count.
2. Cells will be frozen at 5 x 10^6 to 1 x 10^8 cells/mL (cryovials generally are 1 – 2 mL in size).
3. Based upon the number of vials to be prepared and cell number needed, centrifuge the appropriate volume of the cell suspension.
4. Aspirate off the growth medium.
5. Resuspend in freezing medium at 5 x 10^6 to 1 x 10^8 cells/mL.
6. Place vials in automatic freezing unit. If no such device is available, place vials on ice for about 1 – 2 hours, move to -20 °C freezer for several hours, next move -70 °C freezer for several hours and finally into liquid nitrogen storage.

Thawing

1. Retrieve the cells from the freezer and quickly place it into a 37 °C water bath and shake the vial. The cells must thaw quickly as the temperature from -50 °C to 0 °C has the greatest potential for cellular damage, so you want to have the temperature move quickly through that temperature range.
2. Remove cells from vial and slowly add growth medium until cells are at appropriate density for plating.
3. Plate in appropriate growth vessel.

In some cases, DMSO may be toxic to cells. For these, centrifuge cells for 2 minutes at 100 xg. Discard the freezing medium and resuspend the cells in fresh growth medium.

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