Freezing and Thawing Frozen Cells

A. FREEZING

An established cell line is a valuable resource and its replacement is expensive and time consuming. It is vitally important that they are frozen down and preserved for long-term storage. As soon as a small surplus of cells becomes available from subculturing, they should be frozen as a **seed stock**, protected, and not be made available for general laboratory use. **Working stocks** can be prepared and replenished from frozen seed stocks. If the seed stocks become depleted, cryopreserved working stocks can then serve as a source for preparing a fresh seed stock with a minimum increase in generation number from the initial freezing. The best method for cryopreserving cultured cells is storing them in liquid nitrogen in complete medium in the presence of a cryoprotective agent such as dimethylsulfoxide (DMSO). Cryoprotective agents reduce the freezing point of the medium and also allow a slower cooling rate, greatly reducing the risk of ice crystal formation, which can damage cells and cause cell death.

- Prepare freezing medium and store at 2° to 8°C until use.
- 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 (**DMEM**).
- Centrifuge the cell suspension at approximately 100–200 × g for *10 min*. Aseptically discard supernatant without disturbing the cell pellet.
- Resuspend the cell pellet in cold freezing medium at the recommended viable cell density for the specific cell type.
- Dispense aliquots of the cell suspension into cryogenic storage vials.
- Mix frequently and gently the cells to maintain a homogeneous cell suspension.
- Place the cryovials containing the cells in an isopropanol chamber and store them at –80°C overnight.
- Transfer frozen cells to liquid nitrogen, and store them in the gas phase above the liquid nitrogen.

Materials :

- Culture vessels containing cultured cells in log-phase of growth
- Complete growth medium
- Cryoprotective agent such as DMSO
- Disposable, sterile 15-mL or 50-mL conical tubes
- Sterile cryogenic storage vials (i.e., cryovials)
- Isopropanol chamber → achieves temperature reduction gradually
- Liquid nitrogen storage container

B. THAWING FROZEN CELLS

The thawing procedure is stressful to frozen cells, and using good technique and working quickly ensures that a high proportion of the cells survive the procedure.

- Remove the cryovial containing the frozen cells from liquid nitrogen storage to the laminar flow hood for 1 minute at room temperature. Loosen the caps before.
- Transfer it in a 37 °C water bath and quickly thaw the cells by gently swirling the vial in the 37°C water bath until there is just a small bit of ice left in the vial.
- Transfer the desired amount of pre-warmed complete growth medium (DMEM) appropriate for your cell line dropwise into the vial containing the thawed cells.
- Transfer the suspension to a sterile 15-mL conical tube.
- Centrifuge the cell suspension at approximately 200 × g for *10 min*.
- Aseptically decant the supernatant (DMSO) without disturbing the cell pellet.
- Gently resuspend the cells in complete growth medium.
- Transfer them into the flask and into the recommended culture environment.

Materials :

- Complete growth medium (DMEM), pre-warmed to 37°C
- Disposable, sterile 15-ml conical tubes, tissue-culture treated flasks
- Water bath at 37°C