

## Cryopreservation

Cryopreservation is the technique of freezing cells and tissues at very low temperatures at which the biological material remains genetically stable and metabolically inert, and ice crystal formation is minimized. In general, when a tissue is subjected to low temperature, ice crystals will eventually form. These crystals may disrupt the cell membrane leading to death of the cell.

The goal of cryopreservation is to replace some of the water in the cells with other compounds that will not form large crystals when frozen. The most common replacements are DMSO (dimethyl sulfoxide, such as cellgro<sup>®</sup> 25-950-CQC) and glycerol. These are mixed into a solution with media or serum in which cells are suspended and placed in a liquid nitrogen freezer. As the media begins to freeze, the salt concentration outside the cells will become greater than that in the cells and water will leave the cells to be replaced by the cryopreservative.

Cryopreservation media generally consists of a base medium,

protein source, and a cryopreservative. The cryopreservative both protects the cells from mechanical and physical stress and reduces the water content within the cells, thus minimizing the formation of cell-lysing ice crystals. The protein source, often fetal bovine serum (FBS), also protects the cells from the stress associated with the freeze-thaw process. Cells are frozen slowly, at 1°C/minute, using programmable coolers or by techniques outlined below.

Generally, the optimum cell density to freeze per 1 mL of cell suspension depends on the type of cell. Mammalian cells are usually frozen between  $1 \times 10^6$  cells/mL to  $1 \times 10^7$  cells/mL. The cryopreservation media may differ slightly for adherent and suspension cell types.

The following is a suggested procedure for successfully cryopreserving cells. Any specific requirements should be confirmed before beginning.

### Procedure

- Expand culture to allow for adequate cell density for the desired volume to freeze. Optimum conditions include cells currently in the log phase of the growth cycle approximately 2-4 days after subculturing, depending on the cell type. If working with adherent cells, follow appropriate detachment procedures (see Dissociation of Cell Monolayers Technical Information Sheet.)

- Perform a cell count to determine the number of viable cells and the total cell concentration.

- Centrifuge cells at approximately 200-400 x g for 10 minutes, allowing the cells to form a pellet. During centrifugation, determine the amount of freezing media to prepare.

*Example: If using 1 mL cryovials, divide the total cell concentration by the desired cell density. A  $4 \times 10^7$  cell suspension will yield a total of ten 1 mL aliquots at  $4 \times 10^6$  cells per aliquot. Prepare 10 mL of freezing medium to easily suspend the pellet at the correct cell density.*

$$(\text{cell suspension density}) / (\text{desired freezing density}) = \# \text{ of possible 1 mL aliquots at desired density}$$

- Prepare the necessary volume of cryopreservation media using the following guidelines:

#### ADHERENT CELL TYPES:

90% Fresh medium including protein  
10% DMSO or other cryopreservative

#### SUSPENSION CELL TYPES:

45% Fresh medium including protein  
45% Spent medium  
10% DMSO or other cryopreservative

- Resuspend cell pellet(s) using the cryopreservation media, triturating to ensure a single-cell suspension with as few cell clumps as possible.

- Dispense into the desired number of vials for cryopreservation.

- Immediately transfer the vials to a freezer with a temperature of -20°C for one hour.

- Transfer the vials to a -80°C freezer for 24 hours. Alternatively, a dry ice/methanol slurry using a styrofoam or other insulated box with a cover or lid may be used if a -80°C freezer is not available, as long as a constant -80°C can be achieved.

- After 24 hours at -80°C, cells may be transferred to liquid nitrogen for long-term storage (-196°C)