SOP NUMBER: 001.1
TITLE: Cryopreservation of Cells

Objective:
To freeze living cells in a way that they can be revived for growth at a future time.

I. Materials:

A. Equipment
Biosafety cabinet
Cryopreservation container
Tabletop centrifuge
Freezer –80ºC
Freezer Liquid nitrogen
Hemacytometer with cover glass
Incubator, dual chamber
Microscope, inverted
Pipet-Aid
Water bath @ 37ºC

B. Supplies
Aspirating pipets
2cc Cryovials, internal thread
Nitrile gloves
Plastic Serological Pipettes: 1 ml,2ml,5 ml,10 ml
Polystyrene centrifuge tubes

C. Media and Chemicals
1. Chemicals
DMSO (Dimethylsulfoxide)
Growth medium of cell line to be frozen
Trypan blue stain 0.4%
0.25% Trypsin-EDTA or 0.05% Trypsin-EDTA

Safety Note: DMSO is a powerful solvent that can penetrate synthetics, skin, and rubber gloves. It can carry toxins and infectives quickly into the skin and circulation

2. Freezing media formulation: Growth medium, 10% DMSO
For 50 mls: add 5 mls DMSO to 45 mls of growth medium

II. Method:

1. Remove the flask of cells to be frozen from the incubator.

2. Observe that the cells are healthy, have no signs of contamination, and are growing rapidly. Cells should be growing in "log phase" (confluent cultures). The cells should also have the traits of interest. For example, test B-cell hybridomas for monoclonal antibody titer.

3. Trypsinize adherent cells.

4. Perform cell count using 0.1 ml of cell suspension (remove in a sterile manner with a 1 ml serological pipet) and 0.1 ml of trypan blue. Count a minimum of 200 cells in the hemacytometer (@ 50 cells/square).

5. The cell number should be at least $5x10^6$ cells per ampule to be frozen and have a viability of 90% or greater. If you must freeze cells with lower viabilities (i.e. there's no backups and the original source is no longer available), increase the concentration of cells (e.g. $1x10^7$ cells/ml/ ampule) so that there are more cells available upon thawing.

6. Remove volume of cell suspension to be frozen from tissue culture vessel (T-flask roller bottle, etc.) and place in a sterile conical centrifuge tube. (Place volumes of 15 mls or less in a 15 ml conical tube:

SOP NUMBER: 001.1
TITLE: Cryopreservation of Cells

7. Pellet cells for 5 minutes in centrifuge at 150 x g (1200 rpm) at room temperature .

8. Label cryovials with date, cell line designation, study participant number, name of investigator, cell type (e.g. lymphocyte), and passage number (for all adherent cells).

9. Aspirate medium and resuspend cells in 1 ml of freezing media for each $5x10^6$ cells. Example: we routinely freeze $5x10^6$ cells per vial. A total of $2x10^7$ cells produces 4 vials. The cell pellet will be resuspended in 4 mls of freezing medium with 1 ml of cell suspension per vial.

10. Place vials in cryopreservation container (Mr. Frosty") containing 90% Isopropal alcohol.

11. Place container in –80ºC freezer for 18-24 hrs. Note: Maximum number of days in freezer is three.

12. After 18-24 hrs, transfer the cryovials to the liquid nitrogen freezer. Multiple vials from the same sample should be divided between two different liquid nitrogen freezers.

III. Safety Issues

1. Glass ampules. Imperfectly sealed glass ampules can shatter dangerously during thawing due to leakage of nitrogen into the ampules. Polypropylene ampules are less likely to explode because gas can readily escape. USE FACE PROTECTION WHEN THAWING CELLS.

2. Dimethylsulfoxide (DMSO) see above safety note in Section C.

IV. General Issues in Cryopreservation

1. Cells in plastic ampules take 4 times longer to thaw than cells in glass ampules. with agitation that difference is decreased in half. Cell recovery and viability are nearly identical for cells stored in glass vs plastic ampules.[1]

2. It is possible to freeze whole flasks of attached cells or microtiter plates by growing cells to late log phase and adding 10% DMSO to the media. Place the flask in a polypropylene container with 15 mm thick walls at -80C. Thawed cells should be diluted 1:10-1:20 to reduce the concentration of the cryopreservative.

3. DMSO should be colorless, pure, contamination free, and less than a year old. It may become toxic after extended storage. It should be stored in its original bottle.

V. General References

Freshney, RI. Culture of Animal Cells. 3rd Edition. Wiley and Sons. New York, New York, 1994

Penno, Margaret B. Complete Cell Culture-A Practical Approach. "Cryopreservation", pp 108-112, Johns Hopkins University, Baltimore, Maryland,1999.

[1] Cryobiology 14: 500-502, 1977.