SOP NUMBER: 007
TITLE: Thawing and Plating Cells

Objective:

To thaw frozen cells so that they can be established in culture.

I. Materials:

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

B. Supplies
Aspirating pipets: 2 ml
Cryogloves
Dry ice
Face shield
Nitrile gloves
Plastic Serological Pipettes: 1ml, 2 ml, 5,10 ml
Polystyrene centrifuge tubes with screw cap, 15 ml

C. Media and Chemicals
1. Chemicals
Growth medium of cell line to be thawed
Phosphate Buffered Saline w/o calcium amd magnesium (1x)
Trypan blue stain 0.4%

II. Method:

1. Label 15 ml conical tube with cell line designation. Pipet 10 mls of PBS into each tube.

3. Remove frozen vial of cells from the liquid nitrogen freezer and place on dry ice.

4. Thaw cells rapidly in a 37ºC water bath while swirling. The swirling prevents formation of intracellular ice. **Safety Note:**The operator should wear safety glasses and a face shield.

5. After thawing, slowly add 1 ml of PBS drop-wise to the vial of cells. This is to reduce osmotic swelling upon rehydration and improve post-freeze viability.

6. Pipet the 2 mls of cell suspension from the cryovial and place in the 15 ml conical tube containing PBS.

7. Wash and pellet cells at 150 Xg to remove cryopreservative.

8. Count cells and determine post-freeze viability with Trypan Blue dye. See SOP #290: Cell Count Determination. If post-freeze viability is low, cells should be reseeded at a high concentration to increase chances of recovery.

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9. Establish growth in pre-warmed culture medium that has been tested for sterility.

Reference: Margaret B. Penno, “Cryopreservation” pp108-112, Basic Cell Culture-A Practical Approach, 1998.

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