Appendix XI

RECOVERY OF CRYOPRESERVED CELLS

Generally research use only:

1. Obtain the frozen cells from liquid nitrogen storage and immediately place the vial in a 37^{o}C water bath **WITH A PROTECTIVE COVER. **** Allow to thaw for 2 minutes (no more than 3 minutes).

2. Wipe the outside of the vial with 95% alcohol.

3. Transfer the contents of the vial to a new 125 cm^2 flask using a sterile transfer pipette.

4. Gradually add 30 mL growth medium to the cells (slowly over 2 minutes) to dilute the cells.

5. Incubate at 37^{o}C and observe at 24 hours for cell adherence and growth.

6. Discard old medium and refeed cells with 30 mL of growth medium at 24 hours to remove all traces of DMSO and reincubate the cells.

7. Replace growth medium with maintenance medium when the monolayer is confluent (usually after 2-4 days).

8. Replace maintenance medium with fresh maintenance medium once a week.

Reference

Isenberg, HD: Clinical Microbiology Procedures Handbook. American Society of Microbiology, 1992. Pg. 8.20.7.