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Section: Virology Manual	Subject Title: Appendix X Cryopreservation of Cell Cultures	
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Appendix X

CRYOPRESERVATION OF CELL CULTURES

Generally research use only:

1. When a new bottle of cells to be frozen arrives, transfer 30 mL aliquots of cells into 125 cm² flasks (about 3 flasks).
2. Incubate at 36°C until a complete monolayer is obtained (approximately 4 - 5 days).
3. Follow steps 1 - 5 in Appendix IX (Trypsinization and maintenance of monolayer cell cultures).
4. Transfer the trypsinized monolayer to a sterile 15 mL centrifuge tube.
5. Centrifuge at 2000 rpm (700 x g) for 10 minutes.
6. Discard the supernate and resuspend the cells in 10 mL cell freezing medium.
7. Distribute 1.0 mL volume into freezing vials labelled with cell line information.
8. Place the vials into a "Mr. Frosty" freezing container and hold in -70°C freezer for 2 - 3 hours.
9. Record in the computer Freezer Program details of the cell lines stored. Use study "CEL".
10. Transfer the vials to liquid nitrogen freezer.

Reference

Diagnostic Procedures for Viral, Rickettsial and Chlamydial Infection. A.P.H.A. 1989. Sixth Edition. Pg. 72.