



LaCell's "How To" Protocol 103
How Do I Cryopreserved Culture Expanded Cells from LaCell?

Written by: LaCell Staff
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1. All personnel should be trained and certified by the Principal Investigator regarding Universal Precautions and Handling of Bloodborne Pathogens.
2. All procedures should be conducted by investigators using appropriate personal protective equipment at all times. Any waste materials should be decontaminated (bleached) and disposed of using appropriate biohazard waste containers.
3. Purchase LaCell Catalog # hASC-01D or equivalent cryopreserved primary cell product.
4. Thaw and seed the cryovial of hASC-01D as described in LaCell Protocol 101.
5. Harvest cells as described in LaCell Protocol 102.
6. Count the total number of cells obtained in the final volume of re-suspended medium. It is anticipated that a confluent T175 flasks will yield between 3 to 10 X 10⁶ cells depending on the donor demographics and passage number.
7. Centrifuge the total volume of cells in either a 15 ml or 50 ml conical tube, depending on the volume and cell number, for 5 minutes at room temperature and 1,200 rpm (300 X g).
8. Return the centrifuge tube to the BSL2 biological safety cabinet and aspirate the supernatant. Take special care not to disturb the pellet.
9. Determine the total number and resuspend pellet with 1 ml of LaCell's Cryopreservation Medium (LaCryoM) for every 10⁶ cells.
10. Aliquot 1 ml containing 10⁶ cells in LaCryoM to individual 1.5 ml or 2 ml cryovial tubes. Perform this step expeditiously to minimize the time between the addition of LaCryoM and the freezing step.
11. Seal cryovial lids tightly (note: leakage of liquid nitrogen into the tubes can spread contaminants between vials under storage and can increase the danger of explosions when the liquid nitrogen evaporates inside the closed container).
12. Transfer cryovials into a Mr. Frosty™ container.
13. Transfer the Mr. Frosty™ container to a -80° C freezer and leave overnight.
14. The following day, transfer cryovials to a liquid nitrogen dewar for long term storage until needed.

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