LaCell's "How To" Protocol 101
How Do I Thaw Cryovials of Cells from LaCell?

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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. Take the cryovial of hASC-01D out of the dry ice container.
5. Use immediately or store in liquid nitrogen dewar until ready for use.
6. Wear protective eyewear. Theoretically, liquid nitrogen can leak into the container and can cause the vial to explode when it sublimates.
7. Thaw individual vials by agitating for 1 minute in 37° C water bath. (Note: Do not process more than 2 vials at any one time!)
8. Wipe the exterior of the vial with 70% alcohol.
9. Transfer the vial to the interior of a BSL2 biological safety cabinet.
10. Open the cryovial and transfer its contents aseptically to a 15 ml conical tube containing 5 ml of LaCell's Catalog # LaSM (Stromal Medium).
11. Centrifuge the 15 ml conical tube for 5 minutes at 1200 rpm (300 X g) at room temperature.
12. Return to BSL2 biological safety cabinet and aseptically aspirate supernatant.
13. Suspend pellet of cells in a volume of 1 ml of LaCell's Catalog # LaSM (Stromal Medium).
14. Using aseptic technique, remove a 10 µl aliquot of cells.
15. Add the cells directly to a 10 µl aliquot of trypan blue solution.
16. Place 10 µl of cell/trypan blue mixture onto a hemocytometer.
17. Count cells in each of 4 different squares. (Note: You should be counting from between 50 to 100 cells per square).
18. Count the number of viable cells (yellow) and non-viable cells (blue) separately and add together to get total cell numbers.
19. Determine the percentage of viable cells using the following formula: Percentage viable cells = (number of viable cells) divided by (viable + non-viable cell numbers).

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20. Determine the number of cells by the following formula: Total number of cells per ml = (viable + non-viable cell number) X (dilution factor of 2) X 10^4 X (final resuspension volume of cells or 1 ml). For example, if you count an average of 45 live cells and 5 dead cells per square, the total number of cells per square is 50 and the percentage viability is 90%. The total number of cells per ml will be 10^6 (100 X 10^4) of which 900,000 will be alive.
21. Plate the primary cells at a density of 10^2 to 3 X 10^4 per square centimeter, depending on your experiment, feeding them with LaCell's LaSM (Stromal Medium). Feed the cells every second day or three times per week (Monday, Wednesday, Friday) until they reach the desired degree of confluency.