LaCell's "How To" Protocol 102
How Do I Harvest Adherent Cells from LaCell?

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Date: July 9, 2015

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. After plating the primary cells onto a flask or plate at a density of 10^2 to 3 X 10^4 per square centimeter, depending on your experiment, feed them with LaCell's Stromal Medium (Catalog # LaSM) every second day or three times per week (Monday, Wednesday, Friday).
6. When the cells reach the desired level of confluence based on microscopic examination, place the cell container in a BSL2 biological safety cabinet and aspirate supernatant using aseptic technique.
7. Rinse the flask or plate of cells twice with pre-warmed (37^o C) LaCell's 1X Phosphate Buffered Saline (Catalog # LaPBS1X).
8. Add 0.05% trypsin/EDTA to the flask or plate using 1 ml for roughly 40 to 50 square centimeters of surface area (4 ml for a T175 flask, for example).
9. Incubate flask or plate in a 37^o C incubator with 5% humidity for 5 to 10 minutes or sufficiently long enough to allow individual cells (not clumps of cells) to de-adhere from the surface.
10. Return the flask or plate to the BSL2 biological safety cabinet.
11. Add a volume of LaCell's Stromal Medium (Catalog # LaSM) to the volume of 0.05% trypsin/EDTA to each flask or plate. Pipet the medium multiple times aseptically to collect all de-adherent cells. Collect total volume in a 15 ml or 50 ml conical tube depending on the total volume.
12. Centrifuge the cells for 5 minutes at 1,200 rpm (300 X g) at room temperature.
13. Return conical tube to the BSL2 Biological Safety Cabinet.
14. Aspirate the supernatant while carefully maintaining the cell pellet.
15. Resuspend the cell pellet in 1 or more ml of LaCell's Stromal Medium (Catalog # LaSM); aim for a cell concentration of 1 to 2 X 10^6 cells per ml.
16. Take an aliquot of 10 µl of cell suspension and add 10 µl of trypan blue solution.

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17. Place 10 µl of cell/trypan blue mixture onto a hemocytometer.
18. Count cells in each of 4 different squares. (Note: You should be counting from between 50 to 100 cells per square).
19. Count the number of viable cells (yellow) and non-viable cells (blue) separately and add together to get total cell numbers.
20. Determine the percentage of viable cells using the following formula: Percentage viable cells = (number of viable cells) divided by (viable + non-viable cell numbers).
21. 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.
22. Suspend the cell suspension at the desired cell concentration for re-plating to flasks or centrifuge at 1,200 rpm (300 X g) for 5 minutes at room temperature in preparation for cryopreservation or other procedures.