LaCell's "How To" Protocol 104
How Do I Induce Adipogenesis in Cells from LaCell?

Written by: LaCell Staff
Date: July 10, 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. 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 10^6 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 at 1,200 rpm (300 X g).
8. Return the centrifuge tube to the BSL2 biological safety cabinet and aspirate the supernatant. Take special care to not disturb the pellet.
9. Determine the desired density of cells and resuspend pellet in LaCell's Stromal Medium (LaSM) at desired concentration.
10. Plate cells at a density of 5 to 30 X 10^3 cells per square centimeter of surface area. Density at the time of plating will determine the length of time in culture before cells reach confluence.
11. Monitor cells in culture expansion until they reach at least 80% confluence. Allow medium to expend (maintain for up to 3 days without changing).
12. Convert culture medium to LaCell's Adipocyte Differentiation Medium (LaADM).
13. Maintain cells in LaADM for 3 days.
14. Convert cultures to LaCell's Adipocyte Differentiation Maintenance Medium (LaAMM).
15. Maintain cells in LaAMM for up to 12 days, feeding every third day.
16. Monitor cells microscopically for the appearance of lipid vacuoles, which appear under phase contrast microscopy as round, yellow globules within the cytoplasm, often surrounding the nucleus.

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