



LaCell's "How To" Protocol 106  
How Do I Induce Osteogenesis 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  $10 \times 10^6$  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  $30 \times 10^3$  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 Osteogenic Differentiation Medium (LaODM).
13. Maintain cells in LaODM for up to 28 days, feeding with fresh LaODM every third day in culture.
14. Monitor cells microscopically for appearance of areas of mineralization, which appear under phase contrast microscopy as densely packed areas of cell growth resembling small mounds surrounded by a monolayer of cells.

**LaCell LLC, New Orleans BioInnovation Center, Suite 304**  
**1441 Canal Street, New Orleans LA 70112**  
**(504) 598-5246**